AD_____

Award Number: DAMD17-98-1-8659

TITLE: Advanced Cancer Detection Center

PRINCIPAL INVESTIGATOR: John Ruckdeschel, M.D., Jeffrey P. Krischner, Ph.D., Rebecca Sutphen, M.D., Nagi Kumar, M.D., Melvyn Tockman, M.D., Ph.D., Richard Jove, Ph.D., Carlos A. Muro-Cacho, M.D., Ph.D., Q. Ping Dou, Ph.D.

CONTRACTING ORGANIZATION: University of South Florida
Tampa, Florida 33620

REPORT DATE: October 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2000	Annual (1 Oct 99 - 30 Sep 00)

4. TITLE AND SUBTITLE
Advanced Cancer Detection Center

5. FUNDING NUMBERS
DAMD17-98-1-8659

6. AUTHOR(S)
John Ruckdeschel, M.D., Jeffrey P. Krischner, Ph.D., Rebecca Sutphen, M.D., Nagi Kumar, M.D., Melvyn Tockman, M.D., Ph.D., Richard Jove, Ph.D., Carlos A. Muro-Cacho, M.D., Ph.D., Q. Ping Dou, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of South Florida
Tampa, Florida 33620

E-MAIL:
ccontrol@moffitt.usf.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The goals of the **Advanced Cancer Detection Center** include the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening, and the testing of methods to prevent cancer. The projects included in this report are:

- Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (PI: Tockman)
- Genetic Analysis of Familial Prostate Cancer (PI:Sutphen)
- The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer (PI: Kumar)
- Specific Role of Genistein in Estrogen Metabolism (PI: Kumar)
- Phase IIA Chemoprevention Study of Selenium in Persons at Risk for Lung Cancer (PI: Krischer)
- Development of the Moffitt Cancer Network (PI: Krischer)
- African American Families with Inherited Breast or Ovarian Cancer (PI:Sutphen)
- Molecular Fingerprint of STAT3 Regulated Genes for Early Detection of Human Cancer (PI:Jove)
- The Tampa Bay Ovarian Cancer Study (PI:Sutphen)
- Molecular Predictors of Disease Behavior in Thyroid Cancer (PI:Muro-Cacho)
- Significance of Bax-dependent Apoptosis in Prevention and Detection of Human Prostate and Lung Cancer. (PI:Dou)

Each of these projects is presented as a complete study in the attached materials.

14. SUBJECT TERMS
Advanced Cancer Detection

15. NUMBER OF PAGES
145

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

INTRODUCTION:

The goals of the Advanced Cancer Detection Center of the H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida include the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening, and the testing of methods to prevent cancer. In addition, the Center created and supports education programs to provide increased cancer awareness and has established working collaborations with the James A. Haley and the Bay Pines VA Medical Centers and the MacDill Air Force Base Hospital.

The Center supports individual projects that further its mission. Each project is reviewed for scientific merit by an internal peer group and an external scientific advisory committee. Preference is given to projects that have potential to lead to independent peer reviewed funding. During the prior grant period the ACDC supported seven cancer control research protocols. Four of these studies were continuations of projects begun in prior years and three are studies initiated this year. The continuing studies are:

- *Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers,*
- *Genetic Analysis of Familial Prostate Cancer,*
- *The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer,*
- *The Specific Role of Genistein in Estrogen Metabolism,*
- *Phase IIa Chemoprevention Study of Selenium in Persons at Risk for Lung Cancer,*
- *Development of the Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers, and*
- *African American Families with Inherited Breast or Ovarian Cancer.*

Two of these studies (Selenium and Telemedicine) received independent funding from the Department of Defense in February, 2000. While their progress reports are included here for completeness, they are also found in their entirety as separate reports.

Four new studies were recommended for funding in November, 1999, after scientific review from an internal advisory committee. They are:

- *Molecular Fingerprint of STAT3 Regulated Genes for Early Detection of Human Cancer,*
- *The Tampa Bay Ovarian Cancer Study,*
- *Molecular Predictors of Disease Behavior in Thyroid Cancer, and*
- *Significance of Bax-dependent Apoptosis in Prevention and Detection of Human Prostate and Lung Cancer.*

BODY:

Overview: The H. Lee Cancer Moffitt Center & Research Institute includes a free standing patient care facility with a large inpatient and outpatient capacity, a major research institute consisting of more than 130 scientific members, a free standing Lifetime Cancer Screening Center and a wide array of outreach and educational activities for the general public and select underserved populations. Moffitt Cancer Center's location at the convergence of the University of South Florida's Health Sciences Center and the main campus sets the stage for its conceptual commitment to interdisciplinary approaches to research and patient care. Moreover, it allows the Center to enjoy all intellectual advantages of a matrix center while remaining operationally freestanding. After 14 years, the Cancer Center's mission remains totally focused on "contributing to the prevention and cure of cancer."

The Cancer Center was created by the Florida Legislature in the early 1980s, to meet a clear and compelling need to respond to Florida's "cancer epidemic." Building a major cancer research and treatment center at the University of South Florida in Tampa was largely the vision of H. Lee Moffitt, a state legislator who served as Speaker of the Florida House of Representatives from 1982-84. Construction of the original, 380,000 square foot hospital facility was funded with $70 million from the state's cigarette tax, allowing the Center to open in 1986.

The initial phase of the Cancer Center's strategic plan called for a rapid and substantial deployment of its clinical, financial, and philanthropic resources to develop a true scientific center of excellence. The Center recruited Dr. John C. Ruckdeschel as the Cancer Center's first director in late 1991. In 1992, he began fulfilling that strategic plan, a process that culminated in the awarding of a Cancer Center Support Grant (CCSG) five years later.

The strategic plan's second phase continues the focus on scientific and clinical growth, with a commitment to increase research facilities by over 200,000 square feet, and to prepare to accommodate twice as many patients by 2009. In 1998, the state legislature committed an additional $100 million to finance the construction needed to meet these goals.

Key Milestones 1997-2000:

- Increased peer reviewed funding from $8 million in 1996 to over $14 million in 2000.
- Increased NCI funding from $4 million to $7.9 million.
- Recruited more than 20 new basic and physician scientists.
- Hosted or co-hosted national and international cancer conferences, with a major conference, *New Molecular Targets for Cancer Therapy*, conducted on October 14-18, 2000.

- Opened 45,000 sq. ft. of additional laboratory space, with the help of an NIH construction grant (RR 13592) to complete one floor.
- Successfully competed for two program project grants: "Cancer Drug Discovery: Cell Cycle Control Targets,"P.I.: Said Sebti, Ph.D. (1 P01 CA78038-01); and "Molecular Oncology Program Project," PI: William Dalton, Ph.D., M.D. (1P01 CA82533).
- Increased the number of patients enrolled on clinical trials (all types) from 1,809 in 1996 to more than 3,700 in 1999.
- Became the 17th member of the National Comprehensive Cancer Network.
- Worked with the University of South Florida to develop an Interdisciplinary Oncology Program (Department of Oncology) which will include most of the Cancer Center's faculty and that allows a distinct practice plan arrangement with the USF College of Medicine. This Department includes all the basic science faculty recruited by the Cancer Center and will be the academic home for a new interdisciplinary Ph.D. program in Molecular Oncology developed jointly by the Cancer Center and USF.
- Developed an Intellectual Property Sharing Agreement with the University of South Florida that gives the Cancer Center a percentage of all royalties and licensing fees on products developed by the Cancer Center's faculty.
- Developed an indirect cost recovery program with the University that more fully recognizes costs incurred by the Cancer Center and provides a proportional return to the Cancer Center.
- In September, 2000, the National Cancer Institute recommended renewal of the Cancer Center Support Grant for 5 years and the designation of Moffitt as an **NCI Comprehensive Cancer Center**, effective with its Notice of Award in February, 2001.

Today, the Cancer Center's membership numbers 130 scientists and clinicians who are USF faculty. More than 94 members-in-residence are housed and supported in the Center's facilities and work under the terms of the USF/Moffitt affiliation and faculty support agreements. Other members are based in University departments. The Cancer Center's 1,500 employees support the work of the physicians and scientists. The Center has annual operating revenues of over $130 million yearly, including an $11 million annual appropriation from the State of Florida, research grants totaling more than $22 million overall (direct), philanthropic donations, and institutional commitment from the University of South Florida in the form of faculty salaries and a portion of clinical practice revenues.

The Cancer Center currently supports four scientific programs:

Program	Leader	Members	Funding (Direct)
Molecular Oncology	Richard Jove, Ph.D.	21	$ 4,217,117
Immunology	Julie Djeu, Ph.D.	14	$ 2,723,938
Clinical Investigations	William Dalton, Ph.D., M.D.	58	$ 3,380,173
Cancer Control	Jeffrey Krischer, Ph.D.	39	$ 10,243,524
Non-aligned members & institutional grants	N/A	5	$ 2,521,795

The Cancer Control Research Program is the largest research program at Moffitt with 39 active members and more than $10 million in research funding. The overall goals of the Cancer Control Research Program remain focused on the reduction of the burden of cancer on individuals and society. The goals of the Cancer Control Research Program are translated into specific focused scientific aims that can be summarized as the application of multidisciplinary research to:

Aim 1 Susceptibility	Identify markers that predict increased cancer susceptibility.
Aim 2 Prevention	Evaluate promising interventions directed at the prevention of cancer.
Aim 3 Early Detection	Develop and testing new early detection strategies.
Aim 4 Health Outcomes	Evaluate interventions to improve the quality of life for cancer patients & their care-givers.

These aims are consistent with those of the Advanced Cancer Detection Center and the funding has been utilized to create an infrastructure to promote the goals of the Cancer Control Research Program by:

- Encouraging collaborative research
- Providing funding for studies that can lead to extramural peer-reviewed funding
- Providing core competencies to support Cancer Control investigators

In order to provide an appropriate mechanism to allocate and manage these funds, the Cancer Center created an administrative core, an internal scientific review committee, and an external advisory committee. The administrative core manages the resources and personnel associated with the ACDC funding, and provides liaison with the Department of the Army and the regulatory bodies that oversee the research. The internal scientific review committee conducts a scientific review of the merits of proposed projects and their potential for peer-reviewed funding and makes funding recommendations. The external advisory committee reviews the organizational structure and scientific directions of the Advanced Cancer Detection Center and the progress made by the individual projects.

During this grant cycle, the internal scientific review committee met in November, 1999, and recommended four new projects for funding. The projects are:

Project	Principal Investigator	DoD Approval Date
Molecular Fingerprint of STAT3 Regulated Genes for Early Detection of Human Cancer	Drs. Jove & Lazaridis	June, 2000
The Tampa Bay Ovarian Cancer Study	Dr. Sutphen	Nov., 2000 (Anticipated)
Molecular Predictors of Disease Behavior in Thyroid Cancer	Dr. Muro-Cacho	Aug., 2000
Bax-dependent Apoptosis in Prevention and Detection of Human Prostate and Lung Cancer	Dr. Dou	Aug., 2000

In addition, the project African-American Families with Inherited Breast or Ovarian Cancer, which had been approved in the prior grant cycle, underwent USF IRB re-review for changes in the consent form in February, 2000, and received its final approval from DoD in June of this year. Despite our best efforts, project approvals took between six and eleven months to obtain from the DoD. Most of these delays were due to the fact that the DoD requires human subject review in addition to the review and approval by the USF IRB. The DoD human subjects committee meets monthly and any recommended changes must then be re-approved by the USF IRB before they can be resubmitted to the DoD. Therefore, even the slightest change means a two month delay. The most difficult projects to get approved are those involving genetics.

Detailed progress reports for each of the continuing and new projects are attached as appendices.

KEY RESEARCH ACCOMPLISHMENTS:

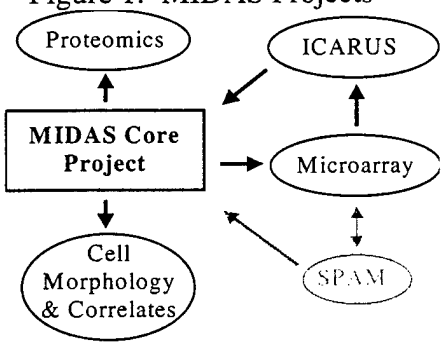
Cancer Control science at the H. Lee Moffitt Cancer Center and Research Institute is greatly enhanced and facilitated by the development of infrastructure that provides access to shared resources, promotes collaboration and funds pilot projects. Over the last three years, the Cancer Control Program has established new infrastructure to meet these needs. The funding of the Advanced Cancer Detection Center is one of three mechanisms by which this has occurred.

1. Advanced Cancer Detection Center

The Advanced Cancer Detection Center has become a significant component of the Moffitt Cancer Control Program infrastructure in that provides a stimulus for research development and promotes inter and intra programmatic collaborations. The Advanced Cancer Detection Center supports pilot studies that can lead to peer-reviewed extramural funding. Projects supported by this mechanism follow a two-tiered scientific review process in which the science and the likelihood of peer-reviewed extramural funding is considered. In addition, priority is given to projects that foster inter and intra-programmatic collaborations.

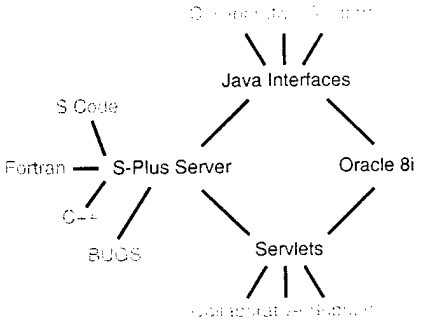
2. Center for Mathematical-Modeling of Image Data Across the Sciences (MIDAS) (PI:Lazaridis), through a two-year competitive grant jointly funded by the Moffitt Cancer Center and the University provost's office in June, 1999. The mission of the MIDAS center is to create interdisciplinary collaborations among imaging, quantitative and biological scientists, in order to develop new analytic models of image-related data and to train researchers of various disciplines in modeling techniques.

Figure 1: MIDAS Projects



The Center is comprised of a core project and several satellite projects. The core project is to construct a data analytic environment that builds advanced statistical methodologies on top of available imaging componentry. By borrowing and uniting technologies from multiple fields, we are seeking to empower researchers in both basic and clinical imaging studies with a sophisticated analytic toolbox. Because this core project is a prerequisite technology for a center focused on quantitative, collaborative analyses of image-related data, substantial resources have been committed to its completion, including hardware, software, and the efforts of two statistical assistants and data systems programmers. Multiple Java interfaces and servlets are being built from reusable object-oriented code to tie together powerful statistical and database systems (Figure 2). Graduate students and postdoctoral fellows participating in the satellite projects also contribute to the core project. New methods have been developed and a patent

Figure 2: MIDAS Core Project Design



application is pending. The applied projects entail interactions with investigators in Molecular Oncology, Clinical Investigations as well as Cancer Control.

3. *Moffitt CCOP Research Base (PI:Krischer)*

The H. Lee Moffitt Cancer Center received funding by the NCI in June 2000 to develop a research base as a mechanism for Community Clinical Oncology Programs to access cancer control clinical trials. NCI CCOPs and Moffitt affiliates are eligible to participate in the Moffitt CCOP Research Base. Membership is based on continued funding as an NCI CCOP with satisfactory performance measured by accrual and data quality.

The goals of the Moffitt CCOP Research Base are to:

- Develop cancer control trials of high scientific merit for implementation in the community setting.
- Provide community investigators an opportunity to participate in NCI-supported cancer control clinical trials.

The following CCOPs have, or are in the process of, establishing formal affiliations with the Moffitt CCOP research base:

Florida Pediatric CCOP, Tampa, FL
Merit Care Hospital CCOP, Fargo, ND
Mount Sinai Medical Center CCOP, Miami, FL
South Texas Pediatric MBCCOP, San Antonio, TX
Baptist Center Research Institute CCOP, Memphis, TN
Cancer Research for the Ozarks CCOP, Springfield, MO
Columbus CCOP, Columbus, OH
Greater Phoenix CCOP, Phoenix, AZ
North Shore University Hospital CCOP, Manhassett, NY
NorthWest CCOP, Boise, ID
Southern Nevada Cancer Research Foundation CCOP, Las Vegas, NV

The material that follows in this section summarizes the key research accomplishments associated with each project and task outlined in the appropriate approved Statement of Work for ACDC approved projects. **A full description of the projects and their progress is appended.**

- ***Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers***
  - Developed an infrastructure to identify, accrue, screen and follow a non-diseased community-dwelling population at high risk for lung cancer.
  - Developed procedures for collection and preservation of sputum specimens for new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.
  - Developed an archive of airways cytologic specimens suitable for evaluation of new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.

- Developed an archive of white blood cells suitable to provide individual control specimens for DNA and RNA.
- Developed a potency assay for MoAb 703D4 immunodetection of hnRNP A2/B1 protein expression.
- Identified a panel of 11 LOH markers for sputum lung cancer screening which identifies 84% of lung tumors (See appendix table III).
- Preliminary evidence does not yet support a helical CT screening-associated stage shift in detected lung cancers.

♦ ***The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer***

Preliminary results of the data collected from the pilot phase of the study are as follows:

- We have recruited twenty one (21) eligible subjects for the genistein/prostate cancer pilot study and all have completed the study. The randomized clinical trial has been initiated and 29 subjects have been recruited and are currently active in the study. A total of eight (8) subjects dropped out of the study as they were unable to tolerate the taste of the product, and two dropped out as they experienced constipation.
- Demographic variables of the pilot study subjects is displayed in the appended report. The average age of this group was 72.3 years, 81% had a family history of cancers, 62% had a history of benign prostate condition, 72% had a smoking history and the average alcohol consumption in the group was 20.6 drinks/month.
- Changes in nutritional intake and weight from baseline to end of study is displayed in Table 2 in the appended report. A slight reduction in caloric and fat intake and an increase in fiber intake was observed. There was an slight increase in weight of 1 lb in this group.
- Changes in proliferative and hormonal markers are displayed in Table 3 in the appended report. Total PSA decreased over 3 points in 62% of the subjects and increased only in 38% of subjects.
- Total testosterone decreased in 65% of subjects and Free testosterone decreased in 50% of subjects. Free estradiol increased in 91% of subjects. However, we failed to see an increase in SHBG levels. On the other hand SHBG decreased in 95% of subjects.
- No abdominal distress, bloating or other gastrointestinal symptoms were reported by the study subjects.

♦ *The Specific Role of Genistein and Estrogen Metabolism*

- Ninety-seven (97) subjects were recruited in the study and sixty six women (thirty-three (33) in each group) completed the intervention.

- Initial comparison of baseline demographic variables such as age, anthropometrics, smoking history, parity, age at menarche, family history of breast cancer and personal history of benign breast disease appears in the appended materials in Table 1.

- Nutritional intake of macronutrients and micronutrients at baseline and post-intervention between the two groups appears in Table 2. Subjects in both groups had similar intake of most macronutrients, micronutrients, including soy isoflavones at baseline. Subjects in both groups were clearly instructed not to consume soy or soy products during the study period. Energy, protein, fat and cholesterol intake significantly increased in the isoflavone supplemented group from baseline to end of study. Upon review of the 4-day food records, it was evident that this increase was a consequence of supplement intake in addition to skim milk or low fat milk that was used to mix the supplement. Protein intake significantly increased in the placebo group from baseline to end of study. As the placebo product was more palatable than the soy supplement, we observed that water or skim milk was used to mix the product.

- The intake of soy isoflavones in the experimental group (Table 3), as predicted, was significantly higher from baseline to post-intervention as compared to the placebo group. The source of this intake was the supplement provided and not from dietary sources. Subjects in both groups were instructed not to consume soy or soy products and other supplements that contain isoflavones during the study period.

- The baseline and final concentrations of serum steroid hormones are displayed in Table 4. The changes in hormonal levels between the two intervention groups was not statistically significant. However, we observed a trend in hormonal changes as hypothesized. SHBG increased in 41.4% of subjects in the experimental group as compared to 37.5% in the placebo group. Free estradiol decreased in 53.85% of experimental subjects compared to 37.5% in the placebo group. Estrone decreased in 55.56% of subjects in the experimental group as compared to 42.86% in the placebo group.

Although changes in steroid hormone levels were not statistically significant, changes in menstrual cycle length were statistically significant (Table 5). Those subjects in the experimental group consuming soy had their mean menstrual cycle increase by 3.52 days compared to a mean decrease of 0.06 days for the placebo group (P=0.04) from baseline to the third menstrual cycle. In addition, subjects on soy had their mean follicular phase increase by 1.46 days compared to a mean increase of 0.14 days for subjects on placebo (P=0.08) (Table 6).

- ***Phase IIA Chemoprevention Study of Selenium in Persons at Risk for Lung Cancer***
  - Established feasibility of recruiting and enrolling heavy current and former smokers on a chemoprevention study.
  - Developed algorithm to recruit and screen subjects, obtain induced sputum specimens, obtain history and physical and screening chest x-ray and blood work prior to bronchoscopy, obtain bronchoscopy and start eligible subjects on selenium supplement.
  - Developed close collaborative relationships with pulmonary medicine and pathology.
  - Evaluated induced sputa from high risk individuals for p16 hypermethylation (all with no hypermethylation detected).
  - Developed archive of 274 bronchial epithelial cell cultures.
  - Developed archive of induced sputum specimens from 151 high risk individuals.
  - Evaluated the value of fluorescent bronchoscopy in addition to white light bronchoscopy in predicting dysplasia in a high risk population (Figure 5).
  - Measured glutathione peroxidase pre- and post-selenium supplementation and found no change.
  - Recent literature report had shown that the levels of a selenoprotein, thioredoxin reductase, increased when exposed to sodium selenite (1). An antibody directed at thioredoxin reductase was used in both western blot and immunohistochemical staining. In the western blot, BEAS-2B cells were treated with either selenomethionine or selenite, but only selenite showed a partial response for a band of the correct size. Unfortunately, the western blot also showed many other bands which would confound any immunohistochemical staining. We did stain slides from two biopsies taken before and after selenium supplementation from four individuals. We saw no obvious difference. We will attempt to find another antibody for thioredoxin reductase that might be useful.
  - Measured selenium pre- and post-selenium supplementation and found increase (dose still blinded)

- ***The Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers***
  - The Moffitt Cancer Network is available to users and can be found at http://network.moffitt.usf.edu
  - The MCN currently has 164 presentations in its library, increasing at a rate of 2.3 presentations per month on average. Additionally, 11 conferences sponsored by USF and Moffitt are also currently available online.

- All approved Grand Rounds presentations have been taped by the Moffitt Multimedia Education Resources Center (MERC) for over one year preceding this report. The video is captured on digital DVCAM 94 minute tapes.
- Since many of the presenters use only 35mm slides for their presentations, a process of creating final production audio/video Real media for streaming via TCP/IP has been developed. This process requires post-production labor and requires the best of the video's individual frames to be captured a second time to recreate higher quality computer images. MCN has made significant progress in this area and as of June 2000 has begun using presenter's PowerPoint files when ever possible to bypass the second image rendering process. This has reduced labor time from 3.5 days to about 5 hours, while increasing image quality noticeably. This labor savings is not realized when presenters are using 35mm film only.
- In addition to pre-presentation file acquisition, MCN has begun the development of a presenter packet. When finished, this packet will inform presenters to repeat important questions asked at the end of events like Grand Rounds and these will be added to the content to be available to medical professionals at the MCN website.
- National oncology conferences have been taped and included in the MCN website database. Conferences have been subdivided into their respective presentations and are categorized searchable as well as searchable using the website database Access Jet engine. All conferences are pre-qualified for their ability to become online educational materials by the University of South Florida College of Medicine and, more recently, the University of South Florida College of Nursing.
- MCN is now beginning to test and research a second media streaming process using MPEG-4. Not standardized by the World Wide Web Consortium yet, the newly introduced streaming format allows for embedded script and control processes within the media stream.

♦ ***Molecular Fingerprint of STAT3 Regulated Genes for Early Detection of Human Cancer***

- Identified 295 unique mouse genes which display altered regulation by v-Src in the context of STAT3 activation and oncogenesis.
- Identified 1045 unique rat genes which display altered regulation by v-Src associated with STAT3 activation during oncogenesis.
- Identified 227 human genes that display altered expression associated with STAT3 activation in breast cancer.
- Identified 64 unique genes that display altered expression resulting from inhibition of STAT3 signaling and thus represent potential STAT3-regulated genes involved in malignant progression of breast cancer.

♦ *The Tampa Bay Ovarian Cancer Study*

Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study, funding was requested and awarded from the American Cancer Society (National) for a companion study to the Tampa Bay Ovarian Cancer Study. The 3-year companion study will evaluate biologically active plasma lysophospholipids and serum CA-125 levels of study subjects (women with newly diagnosed epithelial ovarian cancer), and healthy control women, in order to:

- determine the correlation between plasma lysophospholipid levels and epithelial ovarian cancer stage;
- compare plasma lysophospholipid levels between healthy controls and ovarian cancer patients;
- perform statistical analyses to determine the best correlation with ovarian cancer stage between plasma lysophospholipid levels alone, serum CA125 levels alone or lysophospholipid/CA125 levels in combination;
- determine the correlation between time-based plasma lysophospholipid levels and disease-free survival; and
- assess time-based lysophospholipid measurements as a biomarker by mathematical modeling.

♦ *Molecular Predictors of Disease Behavior in Thyroid Cancer*

- To date, three cases have been collected for the purpose of this study. In all three cases, paraffin-embedded material and frozen sections have been prepared from tumor and adjacent non-neoplastic tissue. Furthermore, in two cases, cell blocks have been prepared from fine needle aspiration biopsy material. This material is being preserved for future testing once all testing conditions are optimized.
- We have also begun the collection of archival paraffin-embedded material from thyroid cancer specimens operated at Moffitt Cancer Center. This material will be used to establish the optimal immunohistochemical conditions for TGFβ type II receptors and the family of Smad proteins. The appropriate antibodies and vendors have been identified. Once conditions are established, approximately 60 thyroid papillary carcinomas will be tested in a prospective manner.
- Results from the immunohistochemistry studies will be correlated with PCR and RT-PCR analysis of the RET oncogene and its transcription products, and also with members of the TGFβ pathway. Nucleic acid material from tumoral and adjacent non-neoplastic cells will be independently collected from tumor frozen sections by Laser Capture Microdissection (LCM). Preliminary studies have been directed to identify optimal conditions for microdissection in paraffin-embedded tissues and frozen sections, and to test the quality of the nucleic acids obtained by PCR and RT-PCR, using the actin gene as a target. These studies are currently under way.
- The specific primers to detect the RET translocations and the Smad family of proteins have been designed and will be tested in the near future.

- ***Bax-dependent Apoptosis in Prevention and Detection of Human Prostate and Lung Cancer***

  - Bax is required for proteasome inhibitor-induced apoptosis in human prostate cancer cells.
  - Resistance of human normal lung cells to proteasome inhibitor treatment is associated with failure of these cells to accumulate Bax to mitochondria.

REPORTABLE OUTCOMES:

➳ *Manuscripts, abstracts, presentations:*

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers

December 8-10, 1998	***International Conference on Prevention and Early Diagnosis of Lung Cancer,*** *Johns Hopkins Lung Project* and *Immunocytochemical Screening for Lung Cancer.* University of Varese and University of Massachusetts Medical School, Varese, Italy.
February 12, 1999	***ALCASE Workshop – Lung Cancer: A Revolution in Care,*** *Technology in Early Diagnosis of Lung Cancer.* Embassy Suites, Tampa, Florida
April 26, 1999	***1999 ALA/ATS International Conference Program,*** *Early Sputum Marker for Lung Cancer (hnRNP).* San Diego Convention Center, San Diego, California
April 30, 1999	***Pharmacology Seminar Program,*** *Detection and Immunostaining of the Lung Cancer Sentinel Cell.* University of Pittsburgh, Pennsylvania.
September 13, 1999	***Advanced Cancer Detection Center,*** *External Advisory Committee.* Moffitt Cancer Center, Tampa, Florida
September 30th to October 3, 1999	***The First International Conference On Screening for Lung Cancer,*** Cornell University, New York
October 9-13, 1999	***Annual Congress of the European Respiratory Society,*** *Dysregulation of the Cell Cycle in Lung Cancer.* Madrid, Spain
October 15-16, 1999	***Molecular Biomarkers Workshop***, Roy Castle Lung Cancer Foundation, Liverpool, England
October 26, 1999	***Screening of Lung Cancer Conference***, Gaithersburg, MD
October 31, 1999	**7th Annual Scientific Assembly of the American Association of Bronchology,** *New Horizons in Cytological Based Early Detection in Lung Cancer.* **Chicago, IL**
February 9, 2000	***International Symposium on Early Detection of Lung* Cancer**, *Molecular Screening Program: Past, Present, and Future.* **Tel Aviv, Israel**

February 27-29, 2000	***International Agency for Research on Cancer, Use of Biomarkers in Chemoprevention of Cancer,*** *Lung Cancer: Intermediate Effect Markers.* **Heidelberg, Germany**
March 20, 2000	***Cahan Lectureship at Memorial-Sloan Kettering,*** *Molecular Screening for Lung Cancer.* **New York, NY**
April 12, 2000	***Early Detection Research Network Site Visit at H. Lee Moffitt Cancer Center & Research Institute,*** *Organization of BeDLAM.* **Tampa, FL**
June 16, 2000	***Wayne State University Cancer Conference,*** *Sputum in 2000: Hypothetical Advantages, Practical Limitations, and Novel Approaches,* **Detroit, MI**
June 22, 2000	***Reducing Lung Cancer Mortality: Actions for the New Millenium,*** *Sputum Based Detection of Preinvasive Lung Cancer,* **Washington, DC**
June 27, 2000	***Roy Castle Lung Cancer Foundation and H. Lee Moffitt Cancer Center, Quest for the Cure,*** *Lung Cancer Screening and Early Detection: Spiral CT Scanning and Molecular Markers,* **Tampa, FL**
July 19, 2000	***H. Lee Moffitt Cancer Center/USF Lung Cancer Conference,*** *Epidemiology and Early Detection of Lung Cancer,* **Coeur d'Alene, ID**
July 19, 2000	***H. Lee Moffitt Cancer Center/USF Lung Cancer Conference,*** *The Management of Pre-Clinical Lung Cancer,* **Coeur d'Alene, ID**
September 12, 2000	***IASLC 9th World Conference on Lung Cancer,*** *Cellular Targeting in the Molecular Diagnosis of Lung Cancer,* **Tokyo, Japan**

The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer

1. **The Specific Role of Genistein in reducing hormonal and proliferative risk parameters in Prostate Cancer**. Kumar NB, Pow-Sang J, Besterman-Dahan, K, Cantor A, Seigne J & Allen K. Proc 10th Annual Research Conference, American Institute for Cancer Research, August 2000.

2. **The Specific Role of Genistein in reducing hormonal and proliferative risk parameters in Prostate Cancer**. Kumar NB, Pow-Sang J, Besterman-Dahan, K, Cantor A, Seigne J & Allen K. Proc. of the 4th Annual Symposium on Predictive Oncology and Therapy sponsored by the International Society for Preventive Oncology; 2000.

The Specific Role of Genistein and Breast Cancer Risk

The preliminary results of this study was presented at the following International and National Scientific Meetings:

1. Proc.of the 4th Annual Symposium on Predictive Oncology and Therapy sponsored by the International Society for Preventive Oncology; Nice, France, 1998,
2. Cancer Control Branch, NCI, Bethesda, MD, 1999
3. Proc American Institute of Cancer Research Annual Meeting, Washington DC, 1999.
4. Kumar NB, Allen K, Cantor A, Shaw G, & Cox CE. The Specific Role of Genistein in Estrogen Metabolism. Proc. of the 3rd International Symposium on the Role of Soy in Preventing and Treating Chronic diseases, October 1999.
5. Kumar NB, Allen K, Cantor A, Shaw G, & Cox CE. The Specific Role of Genistein in Estrogen Metabolism. Proc. of the 4th Annual Symposium on Predictive Oncology and Therapy sponsored by the International Society for Preventive Oncology; 2000.

Molecular Fingerprint of STAT3 Regulated Genes for Early Detection of Human Cancer

An abstract has been submitted to the Oncogenomics meeting to be held in Tucson, Arizona, in January of 2001. This meeting is sponsored by the American Association for Cancer Research and Nature Genetics, and will be one of the major international meetings on the application of microarray gene expression profiling to cancer. The abstract, which is attached as an appendix, will be presented by Dr. Dominic Sinibaldi, who is a postdoctoral fellow bridging the research groups of Dr. Jove and Dr. Lazaridis on this project.

➳ *Patents and licenses applied for and/or issued:*

A patent covering the software developed for the Moffitt Cancer Network, that was developed under the project "*The Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers* ," is being pursued. A formal disclosure has been developed and preliminary work is underway to assess the patentability of the system.

➳ ***Funding applied for based on work supported by this award:***

1. The archive and preliminary data from "**Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers**" provided essential support to our successful NIH application "The Biomarker Development Laboratory at Moffitt" (NCI-CA 84973, M. Tockman, PI, 1st year/Total award $413,720/$1,903,827).
2. Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study supported by this award, funding was awarded by the American Cancer Society for a 3-year companion study to evaluate the role of biologically active lysophospholipids for their potential as biomarkers of ovarian cancer.
3. *The Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers (DAMD 17-00-1-0004), and Chemoprevention of Lung Cancer with Selenium (DAMD 17-00-1-0062),* received independent funding from the Department of Defense in February, 2000.

CONCLUSIONS:

The Advanced Cancer Detection Center has been a great success. It has attracted quality research projects from among Cancer Center members, it has promoted inter and intra programmatic research and its projects have begun to lead to peer-reviewed extramural funding. In the case of the genistein and lycopene trials, ACDC funding has led to randomized phase III trials that will be supported by the NCI through the CCOP Research Base mechanism. The *Tampa Bay Ovarian Cancer Study* funded by the Center has established the population to conduct the lysophospholipids study that is funded by the American Cancer Society (National). Also, *The Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers* (DoD Cohort Study) is the only study in the nation that currently evaluates both molecular airways markers and helical CT examinations simultaneously in the prospective detection of lung cancer. Until the Cornell and Mayo studies begin their collection of sputum specimens, no other study addresses the relative merits of these apparently complementary techniques for lung cancer screening. This research question addresses the most common cause of cancer death and the only common cancer for which no screening is available. Finally, archive of radiographs, sputum and blood cell specimens provides an infrastructure for other investigators at Moffitt and across the nation. The recent award to Dr. Jong Park of an NCI Early Detection Research Network grant was based on the availability of the Cohort archive. Similarly, the collaboration with SPORE investigators at Texas Southwestern (Drs. Gazdar and Minna) are based upon the availability of Cohort specimens. The prospective design, innovative methods and careful execution of this study make it valuable scientific contribution. Other studies are at other phases of their development and conclusions are included in the appended material for each.

REFERENCES:

References pertinent to the individual projects are contained in the appended material.

APPENDIX A

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers

Melvyn Tockman, M.D

Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (DoD Cohort Study) Progress Report

October, 2000

Introduction (1999 Reprise)

Two promising and practical screening techniques, computerized molecular analysis of airway cell markers (ACM) and helical computed tomography (CT), are now available to examine targeted populations for the earliest signs of lung cancer. Henschke et al (Lancet, 1999 354:99-105) found that 10% of helical CT-detected noncalcified nodules from 2-5 mm through 21-45 mm contained a primary lung cancer. This is four times the sensitivity of a standard chest x-ray taken at the same time. Our preliminary data (Clin Cancer Res, 1997, 3:2237-46) showed that computerized immuno-detection of up-regulated hnRNP A2/B1 expression in sputum cells detected primary lung cancer in 37 of 45 (82%) cases. This is 8 times the sensitivity of standard sputum cytology obtained at the same time.

It is quite likely that the helical CT and ACM protein expression screening are complementary. The cell type distribution of the detected cancers suggests this. Henschke reported that of the 27 tumors identified by helical CT, 21 (78%) were adenocarcinomas (includes 3 bronchioalveolar carcinomas), 3 (11%) were mixed squamous-adenocarcinoma, 1 (4%) was a squamous and 1(4%) was an atypical carcinoid. In contrast, among 13 second primary lung cancers detected by protein expression screening, 31 % were adenocarcinoma, 23% were squamous, 15% were mixed adenosquamous, 15% were small cell, 8% were large cell and 8% non-lung primary. Evaluation of the extent to which these early lung cancer detection techniques are complementary could only be conducted in a one-arm prospective study such as this one, where every individual is screened by all techniques at the same examination.

Several years ago with David Sidransky at Hopkins, we pioneered the use of microsatellite alterations as clonal markers in the detection of human cancer (Proc Natl Acad Sci USA 1994; 91:9871-75). We have found that microsatellite alteration and LOH on 3p is significantly associated with upregulation of hnRNP A2/B1 (Proc. AACR 1999; 40:140-1). Further, loss at 3p22, the site of gene for the Type II Transforming Growth Factor Beta Receptor is strongly associated with NSCLC. Alteration of the tumor suppressor TGF-β signaling pathway is of great interest in our laboratory. Therefore, we have developed a technique for preservation of sputum morphology and nucleic acids so that (DNA) microsatellite alterations as well as altered TGF-β type II receptor message expression (RNA markers) may be examined in the sputum specimens collected in this study.

The populations of greatest interest for lung cancer screening are the estimated 46 million former smokers in the United States who remain at risk although they have

stopped smoking. While cardiovascular risk resolves on smoking cessation, Wistuba et al. have shown that genetic alteration of airway lining cells observed in current smokers is not reversed in former smokers (JNCI 1997; 89:1336-73). Progression to lung cancer is probably only slowed by removal of the promotional stimuli of smoking. Major medical centers (Beth Israel, MD Anderson, Cancer 1996; 78:1004-10) now report similar numbers of new lung cancer cases from former as from current smokers.

Age and cigarette smoking are not the only risk factors. We have shown that current and former smokers with airways obstruction are at 2-4 fold risk of developing lung cancer compared to non-obstructed smokers (Ann Int Med 1987:106:512-8). A population of obstructed current and former smokers is identified and available through the Respiratory Division of the James A Haley VA Hospital. This population has been selected to initiate the present study comparing the accuracy (sensitivity and specificity) and predictive value for detecting pre-clinical lung cancer by ACM of upregulated gene expression of hnRNP A2/B1 and by helical CT scanning.

Body

Start-up: Study Activation, 0-3 Months

Approval: The protocol, informed consent and data collection forms were completed and this study was approved by Moffitt/USF IRB on November 5, 1998, with conditional approval by Army Regulatory Compliance on December 23, 1998. The protocol was resubmitted with amendments covering novel methods of sputum preservation to the Moffitt/USF IRB and received Army Regulatory Compliance final approval and study activation on June 10, 1999.

Staff Hired: A total of 7.3 FTEs are now working on this project; 3/4 of these (5.4 FTE) were hired to work directly on participant accrual, registration, interview and specimen collection. This distribution of personnel reflects the importance of patient accrual and specimen collection to the success of this study.

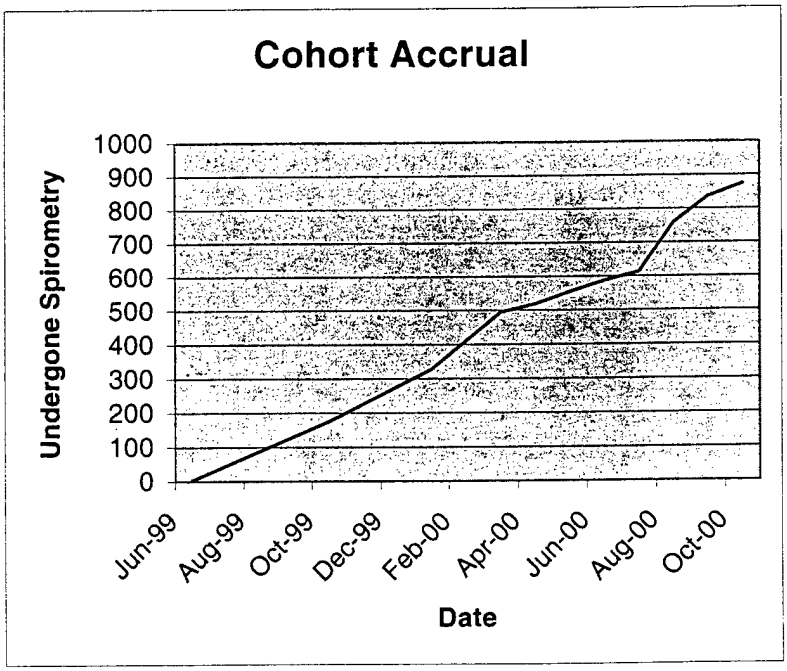
Space Renovation: Two spirometry/sputum induction facilities have been established. One at the LifeTime Cancer Screening Center is fully operational. This facility includes a spirometry screening station, a laminar-flow sputum induction hood, and a biosafety cabinet for sputum specimen processing. Interviews and blood drawing also take place in this space. The screening station at the James A Haley VA Hospital is used primarily for spirometry screening. The patient accrual staff have WOC privileges at the VA Hospital.

Equipment Purchased: Several major pieces of equipment have been purchased to support this study. These include a Helical CT scanner, a Perkin-Elmer 310 gene scanner, and an Arcturus PixCell II Laser Capture Microdissection device.

Initial Recruitment Begins, 3-15 Months

Accrual: The study plan provides that during the first year, 5000 subjects ≥ 45 years of age with ≥ 30 pack years of smoking will be screened by spirometry to identify 1150 subject with mild obstruction ($FEV_1/FVC \leq 70\%$). Mild obstruction would be expected to occur in 23% of these individuals. Our prior sputum/CXR screening trials have shown that in males of this age range with this smoking history, clinical lung cancer will have a 0.7% (7/1000) prevalence and 0.5% (5/1000) annual incidence. In the presence of mild obstruction, the annual lung cancer incidence increases to 1.1% (11/1000) and continues to rise with increasing obstruction. After four years of screening and depending upon the prevalence of obstruction in the study population, therefore, we would expect 44-50 cases of lung cancer (11-13 cases per year).

At this time, 2,875 individuals have been screened, 860 of whom were eligible to undergo spirometry (See chart of Cohort Accrual). Of these, 444 (52%) met obstructive criteria and have proceeded on to sputum induction and helical CT. Half (51%) of the screened population has been referred from the Respiratory Clinic at the James A. Haley VA hospital. This pool of recruits has more ventilatory obstruction than the general population. Beyond the 23% rate of mild obstruction expected from a (non-clinic) population of cigarette smokers, we now find that 52% of our screened population meet the obstruction criterion. By designing the study to include younger, obstructed participants, we are accruing a population at high risk for lung cancer. The observed lung cancer prevalence of 1.8% is 63% greater than expected. If this trend continues, more than 80 cancers would be expected to develop in this population by the end of the study.



To enhance accrual, we engaged McCarthy Medical Marketing and made personnel changes at the end of July, 2000. Appendix II lists the Moffitt Cohort Study work performed by the marketing firm. To date as a result of these efforts and Dr. Tockman's personal appearances, the Commander of the regional American Legion will be including Cohort study information in the newsletter (sent to 500 members) and Verizon communications has agreed to mail an introductory letter to their 11,000 employees.

Radiographic Screening: One hundred fifty-seven (35%) of the 444 screening helical CT scans have shown an abnormality (non-calcified nodule). Twenty-two (4.9%) of these lesions were new or had increased in size. To date, 10 of these positive lesions have been completely evaluated leading to the detection of 8 lung cancers (see table of Cohort Study Results), one lymphoma and one benign scar. The stage distribution (75% greater than stage I) is more advanced than reported in the literature. Self-selection by symptomatic individuals is a recognized source of confounding of prevalence studies. Although none of the lung cancer cases reported symptoms at the time of study enrollment, two of the Stage IV cases did acknowledge symptoms during their subsequent medical evaluation.

Moffitt Cohort Study Results

Design	Actual (as of 10/2/00)
◆Screened with spirometry:5000	◆Screened with spirometry: 860
◆Eligible~screened with CT & sputum:1150 (23%)	◆Eligible~screened with CT & sputum: 444 (52%)
◆Expected positive prevalence screens: 230-460 (20-40%)	◆Positive prevalence screens: 157 (35%)
◆Expected cancers:50 [12-13 per yr.] (1.1%)	◆Cancers: 8 (1.8%)
◆Exp. Stage Distribution: 80% Stage I	◆Stage Distribution: 2 (25%) Stage I 1 (12%) Stage IIB 1 (12%) Stage IIIA 3 (38%) Stage IV

Molecular Airways Markers: Three markers are to be assayed in the sputum of Cohort participants.

hnRNP A2/B1 overexpression. As outlined in the study protocol, ThinPrep monolayer slides are produced from methanol-preserved (PreservCyt) slurries of induced

sputum. Following automated immunostaining (DAKO immunostainer) with monoclonal antibody 703D4 and alkaline phosphatase labeling (LSAB-II, DAKO), individual cells of interest (proplastic, metaplastic and atypical morphologies) are identified by a licensed cytotechnologist. Images of selected cells are acquired at 100 X (Nikon E800 equipped with Princeton Instruments cooled CCD) and quantified automatically for morphologic and densitometric parameters by a workstation running MetaMorph software (Universal Imaging Corp). A discriminant function developed for the Lung Cancer Early Detection Working Group (LCEDWG) Study of detection of second primary lung cancers would be applied to distinguish positives from negatives.

The LCEDWG study has been recently concluded and medical oncologists (Joseph Aisner, M.D. and David Johnson, M.D.) have reviewed all 50 cases of second primary lung cancer recognized by the 14 North American accrual institutions. The ten second primary lung cancer cases originally used to develop the teaching set upon which staining and scoring methods and discriminant function were based were also reviewed. Unfortunately, three of the ten second primary lung cancer cases had been misclassified by the accrual institutions (a metastasis to the lung and lung cancer recurrence). A new teaching set based upon 22 (confirmed) cases and matched controls from the LCEDWG is now being established. Slide preparation methods, immunostaining and quantitation are all being re-established prior to actual preparation and measurement of Cohort specimens.

Loss of heterozygosity (LOH): Sixty three alleles (Appendix III) reported in the literature to be frequently lost in NSCLC or associated with the genes for transforming growth factor β type II receptor (TBR II) or the downstream signaling SMADs 2 or 4 are being examined to confirm their utility in a panel of LOH markers for Cohort specimens. After establishing the PCR conditions (using ^{32}P end-labeling) for the primers at each allele, the primers are applied to archived (non-microdissected) DNA from 43 frozen paired tumor and normal samples. The frequency of microsatellite alterations (LOH or shift) are determined. The most promising markers are confirmed on tumor DNA microdissected from paraffin sections from73 Yunnan Tin Corporation (YTC) miners who developed primary lung cancer. 9 markers from chromosomes 3p, 9p, 9q, and 17p have been selected. Primers for alleles on chromosomes 12p and 18q are being evaluated now. Once selection of the marker panel is complete, the conditions for multiplexed, automated capillary LOH assessment on the PE 310 will be established in preparation for high-throughput assay of Cohort specimens.

Promoter CpG Island Hypermethylation: Tumor suppressor gene transcription may be silenced by promoter CpG island hypermethylation in addition to mutation and allelic loss. Panels of primers for hypermethylation have been recently published and are under study in our laboratory. We have established the conditions for assay of p16 promoter methylation in our laboratory. Four of seventeen (24%) frozen, paired (non-microdissected) DNA specimens demonstrate p16 promoter hypermethylation in our hands. To assure the quality of archived Cohort DNA, 5 Aliquots of DTT/EDTA/DMSO preserved Cohort sputum (1 cancer, 4 noncancers) have been sent to Dr. Adi Gazdar (Texas Southwestern) for assay of methylation of promoter of RASSF1, RAR-beta, p16,

APC, E/H Cadherin. Preliminary results indicate the presence of satisfactorily preserved DNA in all specimens.

Archive: Five hundred and two sputum specimens (includes annual repeats) have been prepared with dithiothreitol (DTT) and EDTA, washed in Hanks solution, spun, resuspended and divided into aliquots for CYTYC Thin-prep slide preparation (for pap staining, immunostaining and storage), in CYTYC PreservCyt (alcohol) slurry and freezing with DMSO in liquid nitrogen. From a similar number of blood specimens, the buffy coats have been separated and stored in liquid nitrogen. Each specimen is bar coded, and computer linked to the database of registration, demographic, medical, smoking, occupational and nutritional history data on each participant.

Database and Lab Specimen Tracking System: Moffitt Cancer Control Research Computing has developed an Oracle database with a Web front-end to allow registration from multiple sites. This database houses the registration, demographic, medical, smoking, occupational and nutritional history data on each participant. Since data entry is still forms-based, the data system was designed to provide easy, intelligent 'double' entry of data. The system has been programmed to provide data constraints, range and referential checks, and edit capability to keep the data clean. The data system provides tools for subject management (generate barcode labels, track unresolved data, report late forms/specimens, etc.). Finally, the relational database will easily provide data for specific queries and statistical analysis. This Research Specimen Tracking (RST) system has now been requested for application to the NCI-SPORE-Lung Cancer Biomarker and Chemoprevention Consortium (LCBCC) study.

Moffitt Cancer Control Research Computing also has developed a Laboratory Specimen Tracking System. This study generates a large number of specimens that must undergo multiple assays in several laboratories. The Laboratory Specimen Tracking System (LST) reads the 2-D specimen barcode to log the specimen into the laboratory. The LST has been programmed to assign each type of specimen a 'profile' that specifies what will happen to the specimen in the lab. A 'profile' consists of a number of steps such as: CheckIn/CheckOut, Assay specimen acceptability, Results Reporting and Archive. The LST is able to track the progress of the specimen and let the lab manager know what step the specimen is on, the specimen turnaround time in the lab, and the archive location of the specimen and its offspring including: Slides, Sputum Slurry Bottles, and Cryovials.

Key Research Accomplishments

- Developed an infrastructure to identify, accrue, screen and follow a non-diseased community-dwelling population at high risk for lung cancer.

- Developed procedures for collection and preservation of sputum specimens for new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.

- Developed an archive of airways cytologic specimens suitable for evaluation of new (DNA, RNA, protein and morphologic) markers of pre-neoplasia.

- Developed an archive of white blood cells suitable to provide individual control specimens for DNA and RNA

- Developed a potency assay for MoAb 703D4 immunodetection of hnRNP A2/B1 protein expression.

- Identified a panel of 11 LOH markers for sputum lung cancer screening which identifies 84% of lung tumors (See appendix table III).

Preliminary evidence does not yet support a helical CT screening-associated stage shift in detected lung cancers.

Reportable Outcomes

Presentations Related to this Study

December 8-10, 1998 ***International Conference on Prevention and Early Diagnosis of Lung Cancer,*** *Johns Hopkins Lung Project* and *Immunocytochemical Screening for Lung Cancer.* University of Varese and University of Massachusetts Medical School, Varese, Italy.

February 12, 1999 ***ALCASE Workshop – Lung Cancer: A Revolution in Care,*** *Technology in Early Diagnosis of Lung Cancer.* Embassy Suites, Tampa, Florida

April 26, 1999 ***1999 ALA/ATS International Conference Program,*** *Early Sputum Marker for Lung Cancer (hnRNP).* San Diego Convention Center, San Diego, California

April 30, 1999 ***Pharmacology Seminar Program,*** *Detection and Immunostaining of the Lung Cancer Sentinel Cell.* University of Pittsburgh, Pennsylvania.

September 13, 1999 ***Advanced Cancer Detection Center,*** *External Advisory Committee.* Moffitt Cancer Center, Tampa, Florida

September 30th to October 3, 1999 ***The First International Conference On Screening for Lung, Cancer,*** Cornell University, New York

October 9-13, 1999 ***Annual Congress of the European Respiratory Society,*** *Dysregulation of the Cell Cycle in Lung Cancer.* Madrid, Spain

October 15-16, 1999 ***Molecular Biomarkers Workshop***, Roy Castle Lung Cancer Foundation, Liverpool, England

October 26, 1999 ***Screening of Lung Cancer Conference***, Gaithersburg, MD

October 31, 1999 **7th Annual Scientific Assembly of the American Association of Bronchology,** *New horizons in cytological based early detection in lung cancer.* **Chicago, IL**

February 9, 2000 ***International Symposium on Early Detection of Lung*** **Cancer**, *Molecular Screening Program: Past, Present, and Future.* **Tel Aviv, Israel**

February 27-29, 2000 ***International Agency for Research on Cancer, Use of Biomarkers in Chemoprevention of Cancer,*** *Lung Cancer: Intermediate Effect Markers.* **Heidelberg, Germany**

March 20, 2000 ***Cahan Lectureship at Memorial-Sloan Kettering,*** *Molecular Screening for Lung Cancer.* **New York, NY**

April 12, 2000 ***Early Detection Research Network Site Visit at H. Lee Moffitt Cancer Center & Research Institute,*** *Organization of BeDLAM.* **Tampa, FL**

June 16, 2000 ***Wayne State University Cancer Conference,*** *Sputum in 2000: Hypothetical Advantages, Practical Limitations, and Novel Approaches,* **Detroit, MI**

June 22, 2000 ***Reducing Lung Cancer Mortality: Actions for the New Millenium,*** *Sputum Based Detection of Preinvasive Lung Cancer,* **Washington, DC**

June 27, 2000 ***Roy Castle Lung Cancer Foundation and H. Lee Moffitt Cancer Center, Quest for the Cure,*** *Lung Cancer Screening and Early Detection: Spiral CT Scanning and Molecular Markers*, **Tampa, FL**

July 19, 2000 ***H. Lee Moffitt Cancer Center/USF Lung Cancer Conference,*** *Epidemiology and Early Detection of Lung Cancer,* **Coeur d'Alene, ID**

July 19, 2000 ***H. Lee Moffitt Cancer Center/USF Lung Cancer Conference,*** *The Management of Pre-Clinical Lung Cancer,* **Coeur d'Alene, ID**

September 12, 2000 ***IASLC 9th World Conference on Lung Cancer,*** *Cellular Targeting in the Molecular Diagnosis of Lung Cancer,* **Tokyo, Japan**

The first International Conference on Lung Cancer Screening was held in October 1999. A group of international experts in imaging, molecular diagnostics, pulmonology, oncology, epidemiology, clinical trial design, statistics, health care policy and patient advocacy met to address the issues central to lung cancer screening. The meeting was co-sponsored by the ACS, the National Cancer Institute, ALCASE, Weill Medical College of Cornell University and other organizations. The conference reviewed currently available data on lung cancer screening and engaged in intensive analyses of the implications with a view to attaining consensus with respect to the main issues surrounding early detection of lung cancer. The conference organizers asked for two reports of this study "Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers." Dr. Robert Clark, Moffitt Director of Radiology described lung cancer screening with helical CT, while Dr. Tockman presented the Moffitt experience with protein expression screening of ACM.

The Moffitt trial comparing ACM and helical CT was recognized as one of three such studies in the nation that was capable of providing insight into definitive clinical trial design considerations. ("The sites at which CT screening is currently being performed in the United States are Weill Medical College of Cornell University, New York NY; H. Lee Moffitt Cancer Center, Tampa FL; Mayo Clinic, Rochester MN."). The conference appreciated that to answer certain questions, an unscreened comparison group was needed to supplement the three ongoing "one-armed" trials. The conference concluded, "comparative populations could be constructed by matching cases (e.g., age, smoking history, tumor classification) from populations currently enrolled in existing large studies or databases (e.g., PLCO screening trial, SEER). Such new methodologic approaches in response to a perceived public health emergency (i.e., 85% lung cancer mortality) may constitute an important precedent for public health research. The results of this innovative approach may guide public policy in formulating lung cancer screening recommendations and save a significant number of lives. As such, these activities merit high priority for creative funding support."

The complete text of the Consensus Statement from the First International Conference on Screening for Lung Cancer may be found in Appendix I of this report.

Funding Received Based Upon Work Supported by this Award

The archive and preliminary data from "**Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers**" provided essential support to our successful NIH application "**The Biomarker Development Laboratory at Moffitt" (NCI-CA 84973, M. Tockman, PI, 1st year/Total award $413,720/$1,903,827).**

Additional Research Opportunities Received Based Upon Work Supported by this Award

At the First International Conference on Screening for Lung Cancer, the principal investigators from Cornell and Mayo joined with me to coordinate our studies of helical CT and airway cell markers to improve our ability to combine our data and strengthen the power of our studies. The results of the combined studies may then be compared to a variety of external studies (eg. Japanese) and control groups (eg. NCI-PLCO trial) (see Consensus Statement).

The procedures and methods of this trial have been solicited by a newly formed consortium of NCI-Lung Cancer SPORE (Specialized Program of Research Excellence) investigators. This Lung Cancer Biomarkers and Chemoprevention Consortium now has preliminary NIH-NCI funding to begin planning for a national trial of helical CT and airway cell markers screening for lung cancer.

Conclusion

The Markers of Transformation in Airways Epithelial Cells from a Cohort of Obstructed Smokers and Former Smokers (DoD Cohort Study) performs two exciting and valuable functions for the Moffitt Advanced Cancer Detection Center. First, it is the only study in the nation that currently evaluates both molecular airways markers and helical CT examinations simultaneously in the prospective detection of lung cancer. Until the Cornell and Mayo studies begin their collection of sputum specimens, no other study addresses the relative merits of these apparently complementary techniques for lung cancer screening. This research question addresses the most common cause of cancer death and the only common cancer for which no screening is available. Second, the archive of radiographs, sputum and blood cell specimens provides an infrastructure for other investigators at Moffitt and across the nation. The recent award to Dr. Jong Park of an NCI Early Detection Research Network grant was based on the availability of the Cohort archive. Similarly, the collaboration with SPORE investigators at Texas Southwestern (Drs. Gazdar and Minna) are based upon the availability of Cohort specimens. The prospective design, innovative methods and careful execution of this study make it valuable scientific contribution.

Appendix I

THE FIRST INTERNATIONAL CONFERENCE ON SCREENING FOR LUNG CANCER

October 1 - 3, 1999

Weill Medical College of Cornell University, New York, NY

Consensus Statement

Summary

Lung cancer kills more individuals than cancers of the breast, colon, cervix and prostate combined. Recent scientific advances create an extraordinary potential to develop a lung cancer screening program that would prevent untimely deaths of vast numbers of current and former smokers who remain at high risk despite smoking cessation. The most promising of these scientific advances are rapid (single breath-hold helical) CT and computerized molecular analysis of airway cell markers (ACM). Each identifies lung cancers much earlier in their development than previously possible with conventional techniques and are likely to be complementary to each other in enhancing early detection of lung cancer. There is compelling evidence that use of these approaches can lead to high cure rates of lung cancer, a disease which currently has a dismal outcome.

The technology to implement these screening approaches currently exists and could rapidly be extended to offer screening to high-risk populations of smokers and former smokers. This creates an urgent need for research to evaluate how these techniques can best be utilized and the magnitude of the benefit they can create in order to allow appropriate public policy decisions about screening for lung cancer.

In December 1998, the International Conference on Prevention and Early Diagnosis of Lung Cancer, sponsored by the American Cancer Society (ACS), the Union International Contre Le Cancer (UICC), the Alliance for Lung Cancer Advocacy, Support, and Education (ALCASE) and other international organizations re-examined the recommendations against screening for lung cancer. The experts concluded that the information leading to these recommendations based on previous screening trials conducted more than two decades ago had a number of limitations and thus comprised an imperfect basis for current health policy. In light of emerging information and the enormous importance of the lung cancer problem, that conference recommended urgent reconsideration of issues surrounding early detection of lung cancer.

In response to this challenge, the first International Conference on Lung Cancer Screening was held in October 1999. A group of international experts in imaging, molecular diagnostics, pulmonology, oncology, epidemiology, clinical trial design, statistics, health care policy and patient advocacy met to address the issues central to lung cancer screening. The meeting was co-sponsored by the ACS, the National Cancer Institute, ALCASE, Weill Medical College of Cornell University

and other organizations. The conference reviewed currently available data on lung cancer screening and engaged in intensive analyses of the implications with a view to attaining consensus with respect to the main issues surrounding early detection of lung cancer.

It was agreed that subsequent to the institutional policy statements not recommending screening for lung cancer, two important developments have occurred. Compelling evidence has continued to emerge over the past decades that resection of early lung cancer has major bearing on survival, and new techniques now provide for distinctly earlier detection of the disease. From this it follows that modern screening for lung cancer would save lives. Beyond this qualitative conclusion, there is an urgent need to learn about the magnitude of this effect.

It was recognized that more than 20,000 people have already participated in studies evaluating the efficacy of CT screening for lung cancer in the United States, Europe, Middle East, and Japan and more than 9000 using ACM. It was agreed that to further quantify the magnitude of the effect, the recommendation to use randomized controlled trials requires serious reconsideration for several reasons. The principal reasons among these are the high cost, long duration of such studies and the rapid advances in technology, together with existing visions of a less expensive and more rapid approach, one already being implemented by ongoing studies.

The conference agreed to form working groups and on a need to reconvene within six months to more closely review the ongoing studies and other interim developments.

Expanded statement

Screening test(s) for lung cancer should be simple, inexpensive, noninvasive, and potentially widely available with a demonstrated acceptable level of sensitivity, specificity, and predictive value. Computed tomography and automated airway cell marker analysis were considered the most promising as well as being complementary (e.g., with regard to detection of central versus peripheral tumors and squamous versus adenocarcinoma histology). Other modalities were also considered but were deemed to either not meet the requirements stated above as they were still too early in their investigative course or more appropriate for diagnostic evaluation. These included: chest radiography (plain; digital, with or without computer-assisted diagnosis; energy-subtraction imaging, with or without such diagnostics), positron emission tomography, electrical impedance tomography imaging, magnetic resonance imaging, fluorescent light bronchoscopy, and CT virtual bronchoscopy.

Screening is only of value when it is linked with appropriate diagnostic interventions and treatment. The conference attendees concluded further

evaluations of lung cancer screening should be conducted within the framework of an overall research program. Such a program would include standardization of diagnostic evaluation and treatment to minimize unnecessary diagnostics, invasive procedures and surgery. Further evaluation of treatment interventions should also be considered. It was decided that all future screening evaluation should be performed in a programmatic setting which includes outcome evaluation, quality assurance, standardized interpretation, diagnostic evaluation, organized reporting and results communications and education for physicians and screenees.

Very promising data from CT screening trials of about 20,000 screening subjects worldwide were presented, underscoring the need to evaluate these tools rapidly. To permit rapid evaluation, an infrastructure must be developed to allow for assessment of future screening modalities. Essential first steps include standardizing protocols, pooling of cohort data, and identifying support mechanisms to ensure long-term clinical follow-up of vanguard populations. **The sites at which CT screening is currently being performed in the United States are Weill Medical College of Cornell University, New York NY; H. Lee Moffitt Cancer Center, Tampa FL; Mayo Clinic, Rochester MN.** Other centers abroad are Muenster University, Muenster, Germany; Hadassah Medical Center, Jerusalem, Israel; the National Cancer Center Hospital, Tokyo, Japan; and Shinshu University, Japan. It was also suggested that additional sites be added in an organized fashion to allow the rapid collection of sufficient screening data to refine and recommend definitive clinical trial design considerations. **To supplement this information, comparative populations could be constructed by matching cases (e.g., age, smoking history, tumor classification) from populations currently enrolled in existing large studies or databases (e.g., PLCO screening trial, SEER). Such new methodologic approaches in response to a perceived public health emergency may constitute an important precedent for public health research. The results of this innovative approach may guide public policy in formulating lung cancer screening recommendations and save a significant number of lives. As such, these activities merit high priority for creative funding support.**

Further research is necessary to determine optimum details for the target population characteristics (age, smoking history, etc.), periodicity of screening, noninvasive diagnostic algorithms after abnormal screening results, and invasive tissue sampling and treatment algorithms. In the conduct of these studies, consideration should be made to bank (store) images as well as certain samples from screened subjects for further study (e.g., sputum, blood, and exfoliated oral cells).

Three possible evaluation strategies were discussed in detail including the strengths, limitations, and implications of a choice of one design compared with the others. The currently recommended entry criteria for enrollment are: current or former (less than ten years since quitting) smokers, age 50 or more, and

healthy enough to withstand thoracotomy (as determined by pulmonary function test). Such entry criteria are critical since an extension to other population groups may require a new trial according to the orthodox view.

The randomized clinical trial (RCT) with lung cancer mortality as its endpoint is the design that offers protection against the unknown influence of suspected biases such as selection bias, lead-time bias, length bias sampling, and over-diagnosis. It was recognized that such a trial could require 80,000 individuals to be randomized to an active population (AP) or a control group (passive population (PP). A baseline screening and four annual repeat screenings with a follow-up period of 8 years after the last screen would be performed. Earliest opportunity for definitive data from such a trial, if it were to begin in 2000, is between 2009 and 2013 (a total of at least 3 rounds with a minimum of 5 years of follow-up). The large sample size is based on an unanticipated, but possibly worst case scenario of low benefit (10% mortality reduction) and high contamination in the PP.

The benefit of such a design is that the intervention would be evaluated without the influence of unknown biases. However, its high financial cost could lead to greater compromises (e.g., unknown threats to power due to erosion of the integrity of the randomization over time, in particular contamination; lack of acceptance of the study conclusion; new technology overtaking the technology under evaluation, a potential lack of participants because of publicity about the presumed superior efficacy of CT).

A RCT using a surrogate endpoint still provides protection against selection bias but provides an earlier answer at lower costs. The surrogate must be a direct goal of screening (e.g., a more favorable stage-shift (TNM), tumor size, % positive nodes, histology) and strongly predictive of mortality. In such a trial, 40,000 subjects would be required. A baseline and four annual repeat screenings would be required with a follow-up period of at least 3 years after the last screen. The earliest opportunity for definitive data, if the trial were to begin in 2000, would be between 2005 and 2008. While issues related to access to control-group endpoints would need to be resolved the benefits of the design include lower cost and more rapidly available data regarding the question of test efficacy. The concern about this approach includes the significant investment in time, the magnitude of potential error which may be difficult to quantify, lack of consensus about interpretation of end results-some policy makers may not accept the end result based on an approach that depends on predictors of mortality.

The third design, the non-comparative or quasi-comparative design, would require 8,000 to 10,000 individuals. It would require a baseline and a single annual repeat. Follow-up for both surrogate and mortality endpoints would be done, but only on the estimated 300 to 400 malignancies. The earliest opportunity for definitive data, if the study were to begin in 2000, would be 2002.

Issues to be addressed would include access to comparison groups (PLCO screening study or older studies) and matching by relevant factors (e.g., histology, age, smoking history). The benefit of this design is that the data would be available more quickly and be obtained at a lower cost.

In summary, it was concluded that:

Ø A RCT with a death endpoint offers the most unbiased answer, but is not embraced with enthusiasm due to costs (mostly time) and the realistic appraisal that precision erodes over time. In some countries, a decision to offer lung cancer screening may require results from a RCT with a mortality endpoint.

ØA RCT with surrogate endpoints is more attractive because of its inherent economy. Workgroup members were troubled by uncertainty that the intermediate endpoints accurately indicated mortality. It was also recognized that if one is willing to accept a study with surrogate endpoints, one should be able to accept a non-comparative study.

ØAt a minimum, the concept that funding for multiple designs should be considered rather than relying only on a single strategy to evaluate lung cancer screening. Further, the urgency of the public health problem warrants an immediate response by health agencies and professional organizations to support organized data collection and evaluation of the potential benefit and costs of all study designs to answer important questions.

ØA non-comparative design could be used, following similar selection criteria. Data should be accumulated from the international sites currently performing helical CT studies and molecular analysis of airway cell markers. Common data collection procedures for all centers should be organized. This effort should be multi-disciplinary in order to measure all end-results (detection data and follow-up) and address harms as well as benefits, including psycho social issues. Models for efficacy and cost-effectiveness of surrogate endpoint and non-comparative designs should be developed. Efforts should be made to seek to answer questions about selection effect, lead time bias, length bias sampling (and overdiagnosis). Realistic estimates of the influence of potential biasing factors may result in greater applicability of alternative designs using comparative data (e.g., National Cancer Institute trial data such as PLCO, SEER registry, etc.)

It was felt that it was important to seek leadership from the appropriate specialty organizations (e.g., American College of Radiology, American College of Pathology, American Thoracic Society) to insure quality assurance for helical CT and computerized molecular analysis of airway cell markers, guidelines for these tests, subsequent interventions, and pathology

Evaluation of screening for lung cancer is critically dependent on accurate pathologic diagnosis of the disease. The majority of CT-detected malignancies

are peripheral adenocarcinomas and, thus, patient specimens will include putative precursor lesions (i.e., pre-invasive lesions, solitary non-invasive non-mucinous bronchioloalveolar carcinomas and small invasive adenocarcinomas as well as occasional central airway squamous lesions). While not all cytologists and surgical pathologists are familiar with the current 1999 WHO/IASLC classification of lung tumors, the diagnosis of lung cancer Is made with a high degree of accuracy. Difficulties in diagnosis are most often related to tumor sampling, the size of the sample and artifacts. The concept of overdiagnosis should not be confused with a false positive diagnosis of lung cancer by pathologists as these are, fortunately, exceedingly rare.

The early detection of lung cancer by helical CT and computerized molecular analysis of airway cell markers provides an important opportunity for radiologic-pathologic and clinical correlation. Since little is known about the clinical course of atypical adenomatous hyperplasia, solitary non-invasive non-mucinous bronchioloalveolar carcinoma, and early phase invasive adenocarcinomas, a single protocol for specimen handling and a central tissue registry are essential. An international panel will be utilized to reach consensus on difficult lesions, and the tissue bank will insure further clinical, radiographic, light microscopic, immunohistochemical and molecular studies of putative precursor lesions and small carcinomas. Through the collection of these lesions we can further our understanding of the biologic behavior of lung cancer.

Appendix II

Moffitt Patient Recruitment Work Summation

Letter writing

A total of eight letters were developed. Each letter was specific for the following target audience:

- Mayor Dick Greco
- County administrator Daniel Kleman
- Benevolent organization (director, administrator, commander)
- Benevolent organization members
- Major employers
- Employees of major employers
- City employees
- County employees

Dr. Tockman requested minor revisions to these letters on August 14th. Completed revisions and new files were e-mailed back to Dr. Tockman the same day.

Research

Benevolent organizations
Names, addresses, and phone numbers were generated for twenty-nine benevolent organizations in the Tampa/Hillsborough county area.

Major employers
Names, addresses, and phone numbers were generated for the top sixteen employers (total employees) in the Tampa/Hillsborough county area.

Minimal Internet research completed for current lung cancer figures and address for county administrator Daniel Kleman.

Communications

The most significant portion of work hours involved calling the specific organizations/companies and locating the appropriate person with the authority to review the materials and grant permission for distribution to members/employees.

Script development
A script was developed for phone contact with organizations to ensure the recipient received a consistent persuasive message. No organizations or companies contacted refused the opportunity to review the recruitment materials.

A list of interested benevolent organizations and major employers was sent to Dr. Tockman.

Lifetime Cancer Screening Pamphlet

Suggestions for revisions to the pamphlet were mailed to Dr. Tockman.

Appendix III

Identification of Panel of Primers for Cohort Loss of Heterozygosity (LOH) Determination

Name	Location	Adjacent gene	Learning Samples Completed	Results (% with MA)	Test Samples Completed	Results (% with MA)
D3S1007	**3p25**	**NA**	**43 paired tumor and normal samples**	**27.9**	**73 YTC tumor samples**	**28.77**
D3S1067	**p21.1-14.3**	**NA**	**43 paired tumor and normal samples**	**22.3**	**73 YTC tumor samples**	**31.51**
D3S1284	p13-12	NA	43 paired tumor and normal samples	9.3	73 YTC tumor samples	10.96
ACTBP2	5p-q	NA	43 paired tumor and normal samples	25.58		
D8S348	q24.13-24.3	NA	43 paired tumor and normal samples	11.63		
D8S320	8p-q	NA	43 paired tumor and normal samples	4.65		
D9S171	**9p21**	**p16**	**43 paired tumor and normal samples**	**18.6**	**73 YTC tumor samples**	**15.07**
D9S242	**q32-33**	**NA**	**43 paired tumor and normal samples**	**11.62**	**73 YTC tumor samples**	**35.62**
D9S753	q22.1-22.3	P16/IFNA?	43 paired tumor and normal samples	9.3		
IFNA	**9p22**	**IFNA**	**43 paired tumor and normal samples**	**20.93**	**73 YTC tumor samples**	**25.4**
D11S488	q24.1-25	NA	43 paired tumor and normal samples	4.65		
D20S82	20p-q	NA	43 paired tumor and normal samples	9.3		
D20D85	20p-q	NA	43 paired tumor and normal samples	13.95		
D17S122	17P12-11.2	NA			73 YTC tumor samples	23.28
CHRNB1	17P12-11	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle)			73 YTC tumor samples	15.1
TP53	**17P13.1**	**TP53**			**73 YTC tumor samples**	**26.02**
D3S1260	3P23-21	TBR II	19 paired tumor and normal samples	5		
D3S1537	**3P24.2-22**	**TBR II**	**19 paired tumor and normal samples**	**26**		
D3S2317	3P24.2-22	TBR II	19 paired tumor and normal samples	5		
D3S647	3P24.2-22	TBR II	19 paired tumor and normal samples	10.5		
D3S1211	3P24.2-22	TBR II	19 paired tumor and normal samples	15.8		
D3S1483	3P24.2-22	TBR II	19 paired tumor and normal samples	15.8		
D3S2319	3P23-21	TBR II	19 paired tumor and normal samples	15.8		
D3S1100	3P22-21	TBR II	19 paired tumor and normal samples	15.8		
D3S1283	**3P24.2-22**	**TBR II**	**19 paired tumor and normal samples**	**26**		
D3S1583	**3P24.2-22**	**TBR II**	**19 paired tumor and normal samples**	**21**		
D3S1619	**3P24.2-22**	**TBR II**	**19 paired tumor and normal samples**	**31.6**		
D3S1266	3P24-23	TBR II	19 paired tumor and normal samples	5		
D3S1227	3P24.2-22	TBR II	19 paired tumor and normal samples	15.8		

Name	Location	Adjacent gene	Status of research-1	Results (% with MA)	Status of research-2	Results (% with MA)
D3S1612	**3P24.2-22**	**TBR II**	**19 paired tumor and normal samples**	**21.1**		
D3S1609	3P24.2-22	TBR II	19 paired tumor and normal samples	10.5		
D3S1611	3P24.2-22	TBR II	19 paired tumor and normal samples	10.5		
D3S1448	3P22-21	TBR II	19 paired tumor and normal samples	5		
D3S1449	3P22-21	TBR II	19 paired tumor and normal samples	NA		
D3S1298	3P24.2-22	TBR II	19 paired tumor and normal samples	15.8		
D12S99	12p13	CDKN1B	Defining PCR conditions			
D12S320	12p13	CDKN1B	PCR conditions established			
D12S269	12p13	CDKN1B	Defining PCR conditions			
D12S77	12p13	CDKN1B	Defining PCR conditions			
D12S358	12p13	CDKN1B	Defining PCR conditions			
D12S1697	12p13	CDKN1B	PCR conditions established			
D12S98	12p13	CDKN1B	PCR conditions established			
D12S89	12p13	CDKN1B	PCR conditions established			
D12S391	12p13	CDKN1B	PCR conditions established			
D18S535	18q12.3	NA	PCR conditions established			
D18S454	18q12.3-q21.1	NA	Defining PCR conditions			
D18S460	18q21	SMAD2	PCR conditions established			
D18S1118	18q21	SMAD2	PCR conditions established			
D18S450	18q21.1	SMAD2	Defining PCR conditions			
D18S470	18q21.1	SMAD2	PCR conditions established			
D18S474	18q21.1	SMAD2/SMAD4	Defining PCR conditions			
D18S1126	18q21.1	SMAD4	Defining PCR conditions			
D18S1110	18q21.1	SMAD4	Defining PCR conditions			
D18S1099	18q21.1	SMAD4	PCR conditions established			
D18S845	18q21.1	SMAD4	Defining PCR conditions			
D18S46	18q21.1	SMAD4/DCC	Defining PCR conditions			
D18S484	18q21.1	DCC	PCR conditions established			
D18S363	18q21.1	DCC	PCR conditions established			
D18S851	18q21.1	DCC	PCR conditions established			
D18S35	18q21.1	DCC	PCR conditions established			
D18S487	18q21.1	DCC	PCR conditions established			
D18S858	18q21.2	DCC	Defining PCR conditions			
D18S64	18q21.1-32	DCC	PCR conditions established			

APPENDIX B

Genetic Analysis of Familial Prostate Cancer

Rebecca Sutphen, M.D.

Genetic Analysis of Familial Prostate Cancer
Principal Investigator: Rebecca Sutphen, M.D.

1. Introduction

This study is a genetic linkage analysis of families in which more than one individual has been diagnosed with prostate cancer, in order to determine whether the prostate cancer in each family appears to be linked to a specific chromosome region (indicating the location of a cancer susceptibility gene). The identification of genes which predispose to development of prostate cancer represents an important step toward early detection and implementation of advanced surveillance and treatment strategies for individuals at high risk for prostate cancer. Men with prostate cancer who were treated at H. Lee Moffitt Cancer Center & Research Institute (HLMCC) and who reported a family history of prostate cancer were identified through the HLMCC Cancer Registry. Of the 133 men identified, 86 had a family history which appeared suitable for the study, defined as (1) at least three affected members with prostate cancer and/or (2) at least 2 affected members with prostate cancer with at least one individual diagnosed at age 55 or younger.

2. Body

The study received institutional review board approval in June 1998. A Certificate of Confidentiality from the National Institute of Mental Health was obtained in September 1998, and subjects were recruited thereafter. Of the 86 men identified through HLMCC Cancer Registry data, 39 who reported at least 3 affected family members were initially invited to participate. 17 subjects were enrolled in the study prior to January 7, 1999, when the study was interrupted to conduct DOD review. The study was reopened to enrollment March 2, 1999. Of the 39 men initially invited to participate, one individual had died. Six individuals declined to participate due to poor health. Eight individuals agreed to participate, but did not complete enrollment. Seven individuals were unavailable through phone contact.

On April 9, 1999, letters of invitation were mailed to the remaining 47 men from the original set of 86 patients. Telephone contact of this group was attempted beginning in April, 1999. At the same time (April - September, 1999), based on review of family history provided by enrolled subjects, for families with sufficient living relatives to provide blood samples for laboratory analysis, telephone contact of family members was attempted. Unfortunately, in only one family were blood samples available from a sufficient number of family members for inclusion in the laboratory phase of the study (DNA linkage analysis). Therefore, laboratory analysis was not performed. No additional work was performed on this study after September, 1999.

During the recruitment phase, problems encountered included death or ill health of affected family members, making them unavailable for blood sampling. Although cancer registry data provided family history of prostate cancer, it did not provide vital status of affected family members, resulting in far fewer families suitable for study than originally anticipated. It was also noted that subjects who

were more recently diagnosed and undergoing treatment for prostate cancer were more interested in participation. For this reason, it appears that prospective ascertainment of familial prostate cancer cases is preferable to retrospective ascertainment through cancer registry records, although prospective ascertainment will require participation from additional centers in order to accrue sufficient subjects/families. Recommendations for further study include prospective ascertainment of subjects and recruitment from additional centers. In order to accomplish this, we have submitted this work for three years of funding to the NCI-sponsored H. Lee Moffitt Cancer Center CCOP Research Base. If blood samples can be obtained from sufficient family members in at least 10 families, laboratory analysis will be performed.

3. Key Research Accomplishments

4. Reportable Outcomes

We have submitted this work for additional funding through the NCI-sponsored H. Lee Moffitt Cancer Center CCOP, in order to accrue sufficient subjects/families for laboratory analysis.

5. Conclusions

The identification of genes which predispose to development of prostate cancer represents an important step toward early detection and implementation of advanced surveillance and treatment strategies for individuals at high risk for prostate cancer. Work thus far has demonstrated that recruitment of families with multiple living, affected members is labor- and time-intensive. Utilization of a prospective, multi-center recruitment effort will facilitate this important research.

APPENDIX C

The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer

Nagi Kumar, M.D.

The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer

DOD US Army- Advanced Cancer Detection Center Grant)

Introduction:

Several natural anticarcinogens have now been identified in soybeans, such as: protease inhibitors, phytate, phytosterols, saponins, lignans and isoflavones.(14-15) However, our focus will be on the components solely unique to soybeans, the isoflavones, also referred to as phytoestrogens, due to their similarity to estrogen both structurally and functionally. Among the isoflavones, diadzein and genistein are the major forms present in soybeans. After structural modifications by intestinal bacteria, isoflavones are converted to compounds which possess weak estrogenic and anti-estrogenic properties.(16-17) The chemopreventive agent, Tamoxifen, which has both estrogenic and anti-estrogenic properties is structurally related and may act in much the same way as isoflavones. These substances have been shown to not only influence hormonal metabolism but also intracellular enzymes, protein synthesis, growth factor action, cell proliferation and angiogenesis. (14-15,18) Genistein has received a great deal of attention due to it's interesting antiproliferative, estrogenic and anti-estrogenic effects. (19-22) Genistein also showed the highest concentration of all phytoestrogens present in the urine of Japanese men and women consuming their typical diet which is rich in soy products. (23) In a recent review of research regarding the effect of genistein on in vitro and in vivo models of cancer it was found that in 74% of the studies using animal models, the proliferation of mammary and prostatic tumors was significantly reduced with genistein. (24-26) In vitro, genistein also had an inhibitory effect on human tumor cell lines. (24) In another study of plant estrogens on estrogen sensitive cancer cells, genistein was found to compete with estradiol binding to estrogen receptors. It has also been postulated that plant lignans and isoflavonoid phytoestrogens may decrease aromatase activity, a cytochrome P450 enzyme thus decreasing conversion of androgens to estrone and estradiol, which may then play a protective role in the development of hormone related cancers.(27) Although genistein has many interesting anticarcinogenic properties we intend to focus on its the effects specific to hormone metabolism and biological activity specific to prostate carcinogenesis. Phytoestrogens found in soy products have been shown to increase serum SHBG via increased hepatic synthesis which, as a result, then decreases the bioavailability of testosterone. (16,28-30) In addition, although phytoestrogens have been shown to have an antiestrogenic effect in a high estrogenic environment, it has been postulated by at least one researcher that they exert a proestrogenic effect in a low estrogenic environment. We postulate that genistein can theoretically increase production of SHBG by the liver, bind to biologically active testosterone, thus lower free testosterone levels and its bioavailability to target prostatic cells, and with its proestrogenic property in the low estrogenic environment increase the levels of estradiol, thus producing the estrogen/androgen synergy that is essential for protection from prostate cancer, and wish to initially observe serum biomarkers of free testosterone, estradiol,

and sex-hormone binding globulin levels in addition to markers of prostatic proliferation such as percentage free and total Prostate Specific Antigens.

SIGNIFICANCE: Although there are some epidemiological and several animal studies supporting the cancer preventative qualities of soy products there have been no definitive, prospective clinical studies testing the ***exclusive*** effects of specific isoflavones on biomarkers that are implicated in the promotion of prostate cancer. It is important to determine whether change in sex hormones, specifically serum sex-hormone binding globulin (SHBG), free testosterone and estradiol levels vary with increased intake of genistein. If genistein can increase production of SHBG by the liver, bind to biologically active testosterone, thus lower free testosterone levels and its bioavailability to target prostatic cells, and with its proestrogenic property in the low estrogenic environment increase the levels of estradiol, thus producing the estrogen/androgen synergy that is essential for protection from prostate cancer, we will be able to clarify the action of isoflavones on serum sex-hormone metabolism and its role in the reduction of the biologically active form of testosterone. If increased intake of the isoflavone, genistein alters the sex hormone milieu and the bioavailability to target tissues, this should theoretically reduce or halt proliferation, as observed by changes in prostate-specific antigen. Thus manipulation of the diet by the addition of genistein may reduce further progression of prostate cancer in this population. Based on the results of this study, prophylactic therapies using dietary supplements such as genistein, with practically no side effects, may also be used to for high risk populations and replace the more controversial therapeutic, hormonal supplementation regimens that are currently used for prostate cancer risk reduction.

PURPOSE:

The purpose of this study is to evaluate the individual effectiveness of supplementing a group of grade 1-2 prostate cancer patients with a dietary supplement of the isoflavone, genistein (60mgs/day) in producing a change in risk parameters that are implicated in the promotion of prostate cancer, such as decrease in free testosterone, and increase in sex-hormone-binding globulin and estradiol and decrease in proliferation as indicated by decreasing total and percentage free Prostate Specific Antigens.

ACCOMPLISHMENTS:

As planned and outlined in our Statement of Work, we have accomplished the following:

Task 1: Recruitment and Data Collection:

a. We have pre-screened 28 and recruited 21 patients diagnosed with grade 1-2 prostate cancer who were consecutively admitted to the Prostate Program. This was the recruitment completed for the pilot phase of the trial. All 21 subjects have completed the study. In addition, in the randomized clinical trial, which was initiated after completion of the pilot phase, we interviewed and screened 44 subjects and have recruited 29 subjects. We have thus screened 72 and recruited 50 subjects in this study.

b. Upon eligibility, consent was obtained form all subjects
Upon enrollment, the following baseline information has been obtained from all subjects admitted to the study.

1. Confirmation of the accuracy of eligibility information, including the 4-day diet records and using an initial screening form (Baseline only)
2. Demographic information, personal and medical history, hormonal and reproductive history, exercise, smoking and alcohol use history obtained by an RD using the Epidemiological Questionnaire(Baseline only).
3. Anthropometric measurements such as subject's height, weight, skinfold and circumference measurements (Baseline, week 6 and 12)
4. 30 mL Blood samples will be drawn into heparinized tubes in a non-fasting state at the same time of day, between 7:00 AM and 12:00 AM, for each individual to perform hormonal assays and total and percentage free prostate specific antigens.(Baseline, week 6 and 12). Hormonal assays will include free testosterone, sex-hormone binding globulin and estradiol at baseline, week 6 and at week 12. As there are no previous studies that have established the duration required to demonstrate change in hormonal levels with intake of genistein in males, we adopted the time taken to demonstrate hormonal changes with ingestion of genistein in female populations which is within one menstrual cycle. We had thus established the evaluation point as 12 weeks or 3 months for both the female and male groups. The hormonal assays (radioimmunoassay) will be performed by Quest Laboratories. Blood draws are done by the Cancer Center phlebotomist and processed and shipped using standard procedures for shipping to Corning Nichols, who will perform the radioimmunoassys.
5. A biopsy and digital rectal exam will be performed by the GU program chief/oncologist for all patients entered in the study at baseline(routine),which will determines patient's admissibility to the study.
6. The participant will be provided with a 2-day diet record(TDFR) and instructed on reporting food intake, including weights/measures and methods of preparation of foods consumed using standard food models. (Baseline, weekly)
8. Changes may be anticipated in stool frequency or GI discomfort. A pre-validated Nutritional Symptoms Scale is used to monitor GI symptoms during intake of supplements on a weekly basis.
9. A Participant Tracking Form is used to monitor all activities and variables observed during the study period. Activities of each participant is vital for the study such as use of supplements, compliance to all monitors. This form, will in addition, serve as a checklist to monitor these variables for the Project Dietitians.
10. Quality control procedures for data collection and entry are ongoing.
11. Contact numbers were provided to patients

Task 2: Abstraction of Medical Records Data:

c. We have continued to obtain patient disease related prognostic indicators from medical charts
d. Data entry and quality control procedures have been initiated

e. Follow-up interviews for data collection periods at mid-point and post completion of interventions have been completed for 21 subjects in the pilot phase of the study and is ongoing for the remaining 29.
f. Weekly visits to the cancer center to obtain supplements and submission of monitoring instruments
g. Shipping of completed patient's blood sample for hormonal assays and PSAs are ongoing

At the end of the study we will complete the data analysis. Pooled t-tests will, in addition be used to compare mean changes in intake of other nutrients, body composition parameters and nutritional symptoms at the end of Phase II. Pooled t-tests are justified in this case. Even if the data are only approximately normally distributed, the test is well known to be quite robust with respect to the normality assumption. These tests will be two-sided.

Multivariate repeated measures ANOVA will be performed on each variable with time as the repetition variable and treatment group as class variable. "Time effect", "group effect" and "time by group" interaction will be tested. If this last effect is significant, then "time effect" will only be reported separately for each group and "group effect" will only be reported separately for each time. The need to adjust for multiple testing is somewhat controversial. We will exercise some control over the multiple testing problem by comparing groups at each time point only if the overall ANOVA is significant. We recognize that this analysis may have limited power due to missing data. For that reason the repeated measures ANOVA will be considered a secondary analysis and the methods described previously will be primary.

KEY RESEARCH ACCOMPLISHMENTS:

Preliminary results of the data collected from the pilot phase of the study are as follows:

- We have recruited twenty two (21) eligible subjects for the genistein/prostate cancer pilot study and all have completed the study. The randomized clinical trial has been initiated and 29 subjects have been recruited and are currently active in the study. A total of eight (8) subjects dropped out of the study as they were unable to tolerate the taste of the product, and 2 dropped out as they experienced constipation.
- Demographic variables of the pilot study subjects is displayed in Table 1. The average age of this group was 72.3 years, 81% had a family history of cancers, 62% had a history of benign prostate condition, 72% had a smoking history and the average alcohol consumption in the group was 20.6 drinks/month.
- Changes in Nutritional Intake and weight from baseline to end of study is displayed in Table 2 . A slight reduction in caloric and fat intake and an increase in fiber intake was observed. There was an slight increase in weight of 1 lb in this group.
- Changes in proliferative and hormonal markers are displayed in Table 3. Total PSA decreased over 3 points in 62% of the subjects and increased only in 38% of subjects.

Total testosterone decreased in 65% of subjects and Free testosterone decreased in 50% of subjects. Free estradiol increased in 91% of subjects. However, we failed to see an increase in SHBG levels. On the other hand SHBG decreased in 95% of subjects.

No abdominal distress, bloating or other gastrointestinal symptoms were reported by the study subjects.

REPORTABLE OUTCOMES:

1. **The Specific Role of Genistein in reducing hormonal and proliferative risk parameters in Prostate Cancer**. Kumar NB, Pow-Sang J, Besterman-Dahan, K, Cantor A, Seigne J & Allen K. Proc 10th Annual Research Conference, American Institute for Cancer Research, August 2000.

2. **The Specific Role of Genistein in reducing hormonal and proliferative risk parameters in Prostate Cancer**. Kumar NB, Pow-Sang J, Besterman-Dahan, K, Cantor A, Seigne J & Allen K. Proc. of the 4th Annual Symposium on Predictive Oncology and Therapy sponsored by the International Society for Preventive Oncology; 2000.

CONCLUSIONS:

In the pilot phase of this study, preliminary results indicate that 62% of the subjects decreased total PSA by >3 points from baseline to end of the 12-week period. However the ratio of %Free to total PSA increased only in 23% of the subjects and increased in 53% of the subjects. Decreasing ratios below 19% are more likely to be prostatic carcinoma. Although supplementation with the isoflavone genistein did not produce an increase in serum levels of sex-hormone binding globulin (SHBG) in this pilot trial, we were able to observe a decrease in total testosterone in 65% and free testosterone in 50% of the subjects. In addition, we observed a significant increase in serum estradiol levels in 95% of the subjects in the study, demonstrating that supplementation with isoflavones for a 12-wwek period in this group of patients may produce a pro-estrogenic effect as hypothesized. Synergism between androgens and estrogens may be an important factor in the etiology of prostate cancer. This effect on the active estrogen/testosterone balance may be another potential autoregulatory mechanism for the protective effect of steroid hormones in prostate cancer. Our preliminary results indicate that we were able to increase serum estradiol and decrease testosterone levels thus effecting an estrogen/testosterone balance, which is an important factor in the etiology of prostate cancer.

The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer (N.Kumar)

Table 1-Demographic Variables(Pilot Study) (n=21)

Variables	Means/Percentage
Age (Years)	72.3
Height (Inches)	71
Family History	81%
Hx Benign prostate condition	62%
Hx Smoking	72%
Alcoholic drinks/month	20.6

The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer (N.Kumar)

Table 2- Changes in Nutritional Intake & Weight from Baseline to Post-Intervention (Pilot Study) (n=21)

	Baseline Means	End of Study Means
Calories	1911	1828
Fat (grams)	70.4	62.7
Fiber (grams)	13.7	17.4
Weight (lbs)	195.1	196.1

The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer (N.Kumar)

Table 3- Change in Proliferative & Hormonal Markers(Pilot Study) (n=21)

	%Subjects showing Increase	%Subjects showing decrease	%Subjects showing no change
Total PSA	38%	62%	0%
% Free PSA	56%	44%	0%
Ratio of % PSA To Total PSA	23%	53%	24%
Total Testesterone	35%	65%	0%
Free Testesterone	50%	50%	0
%Free Estradiol	91%	8%	0
SHBG	5%	95%	0

The Specific Role of Genistein in Reducing Hormonal and Proliferative Risk Parameters in Prostate Cancer (N.Kumar)

Changes in Proliferative/Hormonal Markers (Pilot Study) (n=21)

Variables	Baseline Means	Post-Intervention Means
Total PSA NG/ML	11.09	7.25
Ratio of %Free to Total PSA	18.8	14.3
Total Testesterone	473.8	440.4
Free Testesterone	73.25	75.08
SHBG	35.4	29.94

APPENDIX D

The Specific Role of Genistein in Estrogen Metabolism

Nagi Kumar, M.D.

The Specific Role of Genistein in Estrogen Metabolism

DOD US Army- Advanced Cancer Detection Center Grant

Introduction:

This study is based on the research that has focussed on isoflavones, specifically genistein that has weak estrogenic and anti-estrogenic properties, similar to the chemopreventive agent, Tamoxifen. Isoflavonoid phytoestrogens found in soy products have also been shown to increase serum SHBG that then decreases the bioavailability of estrogen and testosterone, since higher level of SHBG result in lowering of free estradiol and free testosterone. This may also be due to the weak estrogenic effect of phytoestrogens, which, like Tamoxifen, stimulate the synthesis of SHBG in the liver. Several studies also indicate that phytoestrogens reduce the bioavailability of estrogens by actually occupying estrogen-binding sites exerting a weak estrogenic effect (anti-estrogenic) decreasing the availability of estrogen receptors to endogenous, biologically active estrogen. In another study examining the effects of a soy protein diet of the menstrual cycle, consumption of 60 gms of soy protein over a 1 month period led to a significant increase in follicular phase length and delay in menstruation, further demonstrating the ability of isoflavones to alter the hormonal milieu with potentially beneficial effects similar to those observed with Tamoxifen. In addition, research on in vitro and in vivo models of cancer it was found that in 74% of the studies using animal models, the proliferation of mammary tumors was significantly reduced with genistein. In vitro, genistein also had an inhibitory effect on human tumor cell lines. This inhibitory effect on in vitro cell cultures are attributed to its influence on tyrosine kinase activity. It appears that genistein acts to impair the signal transduction pathway from tyrosine kinase receptors, a step that is necessary for mitosis of human breast cancer cells. In another study of plant estrogens on estrogen sensitive cancer cells, isoflavones such as genistein was found to compete with estradiol binding to estrogen receptors. It has also been postulated that plant lignans and isoflavonoid phytoestrogens may decrease aromatase activity, a cytochrome P450 enzyme thus decreasing conversion of androgens to estrone and estradiol, which may then play a protective role in the development of hormone related cancers. Although genistein has many interesting anticarcinogenic properties we intend to focus initially on its the effects specific to hormone metabolism and antiproliferative properties specific to breast carcinogenesis.

Although there are some epidemiological and several animal studies supporting the cancer preventative qualities of soy products there have been few definitive, prospective clinical studies testing the ***exclusive*** effects of specific isoflavones on biomarkers that are implicated in the initiation and promotion of breast cancer. It is important to determine whether change in sex hormones, specifically serum sex-hormone binding globulin (SHBG), estrone and free estradiol levels vary with increased intake of genistein. If increased intake of the isoflavone, genistein produces an elevation in serum sex-hormone binding globulin(SHBG) and a decrease in serum levels of free estradiol and estrone, we will be able to clarify the action of isoflavones on serum sex-hormone metabolism and its role in the reduction of the biologically active form of estrogen. If increased intake of the isoflavone, genistein alters the sex hormone concentration, bioavailability or metabolism, manipulation of the diet by adding rather than restricting food may reduce breast cancer risk. In addition, as a result of this study, dietary guidelines (e.g. National

Research Council, 1989) may be refined, and the credibility of such recommendations will be enhanced. Based on the results of this study, prophylactic therapies using dietary supplements such as isoflavones, with practically no side effects, may also be used to replace the more controversial therapeutic, hormonal supplementation regimens that are currently used for breast cancer risk reduction. In addition, studies examining the effect of isoflavone, genistein supplementation, combined with fat reduction and the effect of such a dietary regimen on both body composition, weight and sex-hormones can be examined with respect to hormonal cancer risk reduction.

Purpose:
The main purpose of the study was to evaluate the individual effectiveness of supplementing a group of pre-menopausal, breast cancer free women with a dietary supplement of the isoflavone, genistein (40mgs/day) in producing a change in sex-hormones that are implicated in the initiation and promotion of breast cancer, such as a decrease in serum free estradiol and estrone and increase in serum sex -hormone-binding-globulin levels. In addition, our goal was to observe the possible effects of isoflavone supplementation on menstrual cycle lengths, an increase of which has been implicated in reduction of breast cancer risk.

Accomplishments:
As planned and described in the Statement of Work:

Task I: Start up: printing forms, screening, contact individuals regarding recruitment:
The first two-three months of the funding period was utilized to prepare all instruments, consent form packages and start the recruitment task: This was accomplished successfully through recruiting subjects from the Tampa Bay area by public appeal. advertisements in the Tampa Tribune and in the News Media. We have contacted and screened 303 women to date for this study.

Task II: Recruitment, data collection, Process and construct data files:

a. Of the 303 that we initially contacted, we have screened 260 women for eligibility, and recruited 97 subjects in the study. The predominant criteria that excluded women were perimenopausal status, living outside the Tampa area or consumption of soy or other supplements at first screening.

b. Ninety seven(97) pre-menopausal, breast cancer free, omnivorous women, of all races and ethnicity, between ages 25 and 55, inclusive, at first screening contact, residing in the study area for the entire period of the study after randomization, and providing written informed consent were admitted in the study, of whom 66 have completed the study.

c. An unanticipated drop out rate of 30% was observed, with 31 subjects dropping out of the study. Twenty five (25) subjects withdrew as they were unable to tolerate the palatability or texture of the supplement for the study period, one (1) complained of headaches, one (1) subject became pregnant and four (4) reported diarrhea with the products used in the study. Upon eligibility, consent was obtained form all subjects

Upon enrollment, the following baseline information was obtained from all subjects admitted to the study.

1. Confirmation of the accuracy of eligibility information, including the 4-day diet records and using an initial screening form.

2. Demographic information, personal and medical history, hormonal and reproductive history, exercise, smoking and alcohol use history obtained by an RD using the Epidemiological Questionnaire. The instrument will be administered at baseline.

3. Anthropometric measurements such as subject's height, weight, skinfold and circumference measurements. Anthropometric measurements were obtained during the visit to the clinic to see the RD. Weight and body fat distribution charts are maintained on all subjects for the study period.

4. Blood samples were drawn into heparinized tubes in a non-fasting state at the same time of day, between 7:00 AM and 12:00 AM, for each individual. Thirty mL of blood was taken from subjects during the mid-follicular phase of their menstrual cycle or 4-7 days after the start of menstrual flow. These samples were taken at baseline before intervention and again at the end of the study period. Subjects were instructed to call or page the Project Director on Day 1 of their menstrual cycle. An appointment was scheduled for them to meet with the RD and have their blood drawn.
5. After venipuncture is performed the blood will be centrifuged, the plasma separated, ascorbic acid and sodium azide (0.1% final concentration for both) added. The samples collected from each subject were stored at -20 degrees Centigrade until they are packed in dry ice and shipped to Corning Nichols Institute. Plasma levels of estrone, estradiol and sex-hormone-binding-globulin(SHBG) were determined.
6. The participant were provided with a 4-day diet record(FDFR) (Appendix 6) and instructed on reporting food intake, including weights/measures and methods of preparation of foods consumed using standard food models. Written instructions will accompanied subjects with instructions on how to complete a FDFR. Subjects will be instructed to call the RD if questions arise as to how specific specialty foods need to be documented. The trialists paid special attention to subjects from ethnic backgrounds, specific to the Tampa Bay area. Specific food models, handouts, videos, specific to the ethnic groups that are currently used by the trialists were be used. In addition, ethnic differences in nutritional intake between participants will be dealt with by stratified randomization. The RD made telephone follow-up calls during one of the four days assigned for the first week of the intervention.

7. The trialists and the subjects in the study will be blinded as to the nature of the product. Having been assigned to groups A or B, subjects were instructed and provided information on:
   a. Introduction to the product and mixing instructions which are the same for both the experimental and the control group,
   b. Importance of compliance with and completion of 4-day food records,
   c. Compliance and completion of Nutritional Symptoms Scale,

d. Attendance to clinic by the 2 groups to obtain weekly supplements, self-monitoring tools and to submit to the research staff completed self-monitoring tools and leftover supplements, if any.
e. Compliance to diet without altering or reducing other foods.

8. Changes may be anticipated in stool frequency or GI discomfort. A pre-validated Nutritional Symptoms Scale was used to monitor GI symptoms during intake of supplements.

9. Menstrual histories were obtained from all subjects at baseline. The length of the menstrual cycle, both the follicular and luteal phase were be monitored by use of a commercial kit, First Response Ovulation Predictor (Carter-Wallace, Cranbury, NJ), which will provide qualitative information on when ovulation occurred. Subjects were instructed as to the use of the Ovulation predictor kit. Date of Day 1 of menses was required to be documented by the subjects on the Nutritional Symptoms Scale. The follicular phase will be defined as the first day of menstrual cycle to the day that the luteinizing hormone surge was noted. The luteal phase will be defined as from the day after the luteinizing hormone surge was noted to the day before the onset of the next menstrual cycle. This close monitoring of the menstrual cycles will enable us to observe the possible effects of isoflavone supplementation on cycle lengths.

10. A Participant Tracking Form was used to monitor all activities and variables observed during the study period. Activities of each participant is vital for the study such as use of supplements, compliance to all monitors. This form, will in addition, serve as a checklist to monitor these variables for the Project Director.

e. Follow-up interviews for data collection periods at mid-point and post completion of interventions have been completed for all 66 subjects.

f. Weekly visits to the cancer center to obtain supplements and submission of monitoring instruments has been completed for all 66 subjects.

g. Shipping of completed patient's blood sample for hormonal assays has been completed for all 66 subjects.

TECHNICAL PROBLEMS ENCOUNTERED AND HOW OUR APPROACH WAS MODIFIED:

Supplement: Although the isoflavone genistein and milk powder supplement that we initially received from the manufacturer was taste tested in our pilot study, it was felt by the research group that the product tasted "chalky", had no flavor, with poor palatability, as it had been on the shelf for over 8 months. This presented a concern to the researchers as compliance over 12 weeks will present a challenge to the researchers and study subjects. This was reported to Protein Technologies, who then replaced the entire batch of genistein and milk powder with a vanilla flavor. Consequently, the start date for the study was prolonged by 8 weeks, taking us to the

month of October 1997. Based on our experience and as reported in the literature, there was a consensus among the research team that the months of October, November and December would not be ideal dates to start a dietary intervention study, as subjects were required to consume two packages per day and not alter their usual intake. As these months fall during the holidays, apart from compliance to the supplements, unusually high intake of other nutrients may have seriously affected the results of the study. Thus, our first subject was entered into the study on January 5, 1998.

Timing for completion of hormonal assays: To ensure sufficient funding for baseline and post-intervention hormonal assays, all baseline serum samples are currently labeled, packaged and stored appropriately at the Cancer Center laboratory. When subjects complete the study, their baseline and post intervention samples will be picked up by the laboratory at the end of the study period for each subject. This will eliminate the cost of baseline assays on subjects who may then withdraw from the study. The response to the study from the Tampa Bay area was overwhelming. Thus, although the attrition rate is higher than anticipated (30%), we were able to obtain the numbers needed for the study. All other procedures as established in the original proposal were effective and were implemented.

High drop-out rate:
As we had experienced a drop-out rate of 30% in this study, most of which were secondary to intolerance to the supplements, in the future, we plan to eliminate use of isoflavones in liquid protein. Instead, we plan to use a tablet form of the isoflavone supplements and placebo that are currently available. This will eliminate the high drop-out rate and thus the integrity of the study.

Study Period:
The study period has to be extended for an additional year secondary to the high drop-out rate and failure on our part to plan for holiday months. We have in our previous studies and with our clinical experience observed poor compliance to supplements during the months of October, November and December, as subjects were required to consume two packages per day and not alter their usual intake. As these months fall during the holidays, apart from compliance to the supplements, unusually high intake of other nutrients may have seriously affected the results of the study. In the future, we plan to take this phenomenon into consideration while determining the duration of the study period. We thus lost 6 months of the initial 2-year study period.

Upon completion of the data entry, the following hypothesis were tested:
1. Supplementation with the isoflavone genistein will produce an increase in serum levels of sex-hormone binding globulin (SHBG). This increase will exceed that of a placebo control group. A pooled t test will compare the two groups mean SHBG levels at the conclusion of Phase II.
2. Supplementation with the isoflavone genistein will produce a decrease in serum free estradiol and estrone sulfate. These decreases will exceed that of a placebo control group.
Pooled t tests will compare the two groups' mean estradiol and estrone levels at the conclusion of Phase II.

OTHER RESEARCH QUESTIONS:
1. Does supplementation of diet with genistein affect length of menstrual cycle?

At the conclusion of Phase II, the length of each woman's last menstrual cycle will be recorded. The two groups' mean length of menstrual cycle lengths will be compared with a pooled t test.
2. Will women whose diets are supplemented with genistein alter their nutritional selection of specific other nutrients such as fats, fiber, vitamin A and C ?
Each woman's final 4-day diet record will be analyzed for fat, fiber, vitamins A and C intake. Pooled t tests will be used to compare the two groups for intake of each nutrient.
3. Will supplementation with genistein affect anthropometric parameters such as QI, body fat and body fat distribution measurements?
Mean body fat ratio, QI and body weight changes of the two groups at the conclusion of Phase II will be compared by pooled t tests.
4. Will supplementation with genistein produce nutritional symptoms?
The proportion of women reporting increased stool frequency and GI distress in the two groups will be compared by Chi-square tests. If, for either symptom, the number experiencing it is less than five, Fisher's Exact Test will be used instead.

Pooled t-tests are justified in this case. Even if the data are only approximately normally distributed, the test is well known to be quite robust with respect to the normality assumption. These tests was two-sided.

Multivariate repeated measures ANOVA were performed on each variable with time as the repetition variable and treatment group as class variable. "Time effect", "group effect" and "time by group" interaction were tested. If this last effect is significant, then "time effect" will only be reported separately for each group and "group effect" will only be reported separately for each time.

Key Research Accomplishments:

- Ninety-seven (97) subjects were recruited in the study and sixty six women (thirty-three (33) in each group) completed the intervention.

- Initial comparison of baseline demographic variables such as age, anthropometrics, smoking history, parity, age at menarche, family history of breast cancer and personal history of benign breast disease appears in Table 1.

- Nutritional intake of macronutrients and micronutrients at baseline and post-intervention between the two groups appears in Table 2. Subjects in both groups had similar intake of most macronutrients, micronutrients, including soy isoflavones at baseline. Subjects in both groups were clearly instructed not to consume soy or soy products during the study period. Energy, protein, fat and cholesterol intake significantly increased in the isoflavone supplemented group from baseline to end of study. Upon review of the 4-day food records, it was evident that this increase was a consequence of supplement intake in addition to skim milk or low fat milk that was used to mix the supplement. Protein intake significantly increased in the placebo group from baseline to end of study. As the placebo product was more palatable than

the soy supplement, we observed that water or skim milk was used to mix the product.

- The intake of soy isoflavones in the experimental group (Table 3), as predicted was significantly higher from baseline to post-intervention as compared to the placebo group. The source of this intake was the supplement provided and not from dietary sources. Subjects in both groups were instructed not to consume soy or soy products and other supplements that contain isoflavones during the study period.

- The baseline and final concentrations of serum steroid hormones are displayed in Table 4. The changes in hormonal levels between the two intervention groups was not statistically significant. However, we observed a trend in hormonal changes as hypothesized. SHBG increased in 41.4% of subjects in the experimental group as compared to 37.5% in the placebo group. Free estradiol decreased in 53.85% of experimental subjects compared to 37.5% in the placebo group. Estrone decreased in 55.56% of subjects in the experimental group as compared to 42.86% in the placebo group.

- Although changes in steroid hormone levels were not statistically significant, changes in menstrual cycle length were statistically significant (Table 5). Those subjects in the experimental group consuming soy had their mean menstrual cycle increase by 3.52 days compared to a mean decrease of 0.06 days for the placebo group (P=0.04) from baseline to the 3rd menstrual cycle. In addition, subjects on soy had their mean follicular phase increase by 1.46 days compared to a mean increase of 0.14 days for subjects on placebo (P=0.08)(Table 6).

Reportable Outcomes:

The preliminary results of this study was presented at the following International and National Scientific Meetings:

1. Proc.of the 4th Annual Symposium on Predictive Oncology and Therapy sponsored by the International Society for Preventive Oncology; Nice, France, 1998,
2. Cancer Control Branch, NCI, Bethesda, MD, 1999
3. Proc American Institute of Cancer Research Annual Meeting, Washington DC, 1999.
4. Kumar NB, Allen K, Cantor A, Shaw G, & Cox CE. The Specific Role of Genistein in Estrogen Metabolism. Proc. of the 3rd International Symposium on the Role of Soy in Preventing and Treating Chronic diseases, October 1999.
5. Kumar NB, Allen K, Cantor A, Shaw G, & Cox CE. The Specific Role of Genistein in Estrogen Metabolism. Proc. of the 4th Annual Symposium on Predictive Oncology and Therapy sponsored by the International Society for Preventive Oncology; 2000.

Conclusions:

To our knowledge, our study was the first randomized, double-blinded study to examine the effect of soy isoflavones in a substantial number of subjects, on steroid hormones and menstrual cycle length in Western women. There is a general agreement that hormones are involved in the development of breast cancer. A change in the menstrual cycle length alters the relative duration of mammary epithelial cells in the luteal phase of a cycle during which time the breast cells are more proliferative. Thus the levels of these steroid hormones and menstrual cycle length are recognized risk factors for breast cancer. In general, when cycle length increases, the length of the follicular phase increases more than the luteal phase. As breast cells proliferate 2-3 times more rapidly during the luteal phase than during the follicular phase, an increase in the cycle length and an increase in follicular cycle length, as observed in our study in subjects consuming soy supplements, may theoretically shorten the exposure of the breast epithelia to progesterone in the luteal phase. If this occurs over a long period of time with consistent soy consumption, the relative amount of time during which the breast epithelia is stimulated to proliferate may decrease accordingly, and this may decrease the overall breast cancer risk. Differences in cycle lengths between breast cancer cases and controls and between populations of women in countries with different breast cancer risk are consistent with the theory that menstrual cycle length may moderate breast cancer risk. Our results support the hypothesis that soy consumption may alter circulating ovarian steroid hormone concentrations in premenopausal women and increase menstrual cycle length. Menstrual cycle of women from Western populations ranges from 26-29 days, whereas the average cycle length for Japanese and other Asian women is longer, which may be attributed in part to the lower risk of breast cancer in populations that consume soy in their daily diet. Our study was able to demonstrate that daily soy consumption for a period of 12 weeks can lead to significant changes in menstrual cycles even in Western women.

Although we did not observe mean changes in steroid hormonal concentrations with soy supplementation for a 12-week period, we observed a trend demonstrating increase in SHBG and decrease in free estradiol and estrone in a relatively larger percentage of subjects consuming the soy supplement compared to the placebo group. SHBG increased in 41.4% of subjects in the experimental group as compared to 37.5% in the placebo group. Free estradiol decreased in 53.85% of experimental subjects compared to 37.5% in the placebo group. Estrone decreased in 55.56% of subjects in the experimental group as compared to 42.86% in the placebo group. Previous studies examining the effect of soy isoflavones on steroid hormones in premenopausal women have similarly shown only modest changes. In a pilot study, Lu et al (1996) examined the effects of soy in six premenopausal women for 1 month in a metabolic unit and observed reduction in serum 17 beta-estradiol , progesterone and dehydroepiandrosterone sulfate levels and an increase in menstrual cycle length. Similarly Nagata (1998) et al demonstrated a moderate but not a statistically significant decrease in estrone and estradiol levels in a experimental study with premenopausal Japanese women supplemented with soymilk. However, subjects in the experimental group increased their menstrual cycles by nearly 2 days. In two other studies (Duncan 1999 & Martini et al 1999) no steroid hormonal nor menstrual cycle effects were observed. The sample size in these studies were small and/or the duration of intervention ranged from 1 to 2 months. Thus, we were able to demonstrate that daily soy

consumption for a period of 12 weeks can lead to significant changes in menstrual cycles even in Western women, although significant changes in steroid hormonal levels was not evident during this period.

The Specific Role of Genistein in Estrogen Metabolism (N. Kumar)

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF SUBJECTS IN THE EXPERIMENTAL & PLACEBO GROUPS

VARIABLES	ISOFLAVONE SUPPLEMENTED (n=33)		PLACEBO (n=33)	
	$\overline{X}$	SE	$\overline{X}$	SE
Age, years	41.30	1.03	42.48	.80
Baseline Weight, lbs.	143.80	5.14	147.05	4.48
Height, cm.	164.21	1.17	165.50	1.26
BMI kg/m(squared)	24.12	.75	24.33	.66
Age at Menarche	12.53	.20	12.97	.27
Number of Full Term Pregnancies	1.41	.19	1.45	.21
Birth Control Pill Use (Months)	63.00	12.87	51.38	10.53
Current Smoker	3.1%		9.4%	
Family History of Breast Cancer	56.7%		41.9%	
History of Benign Breast Disease	46.9%		39.4%	

The Specific Role of Genistein in Estrogen Metabolism (N. Kumar)

TABLE 2. NUTRIENT CONSUMPTION AT BASELINE AND END OF STUDY PERIOD OF EXPERIMENTAL & PLACEBO GROUPS

	ISOFLAVONE SUPPLEMENTED (n=33)			PLACEBO (n=33)			
	Base Line	Final Week	P	Base Line	Final Week	P	P*
Energy Kcals	1740	1967	.01	1812	1810	.86	.12
Protein (gm)	72.4	110.3	<.001	72.8	97.2	<.001	.18
Fat (gm)	55.4	63.8	.05	61.2	56.1	.31	.03
Cholesterol (mg)	165.8	221.5	.02	229.4	188.4	.17	.01
Carbohydrates (gm)	238.5	228.9	.50	237.0	220.1	.33	.74
Fiber (gm)	44.6	14.8	.91	15.0	13.0	.08	.26
Alcohol (gm)	4.2	5.2	.53	7.9	5.3	.20	.16
Vitamin A (RE)	1151.1	1131.3	.82	1275.9	1030.4	.35	.56
Vitamin C (mg)	130.1	110.1	.11	118.2	140.9	.64	.36
Vitamin E (mg)	8.5	6.1	.46	5.2	4.6	.47	.59
Thiamin B1(mg)	1.53	1.31	.36	1.3	1.2	.77	.49
Riboflavin B2 (mg)	1.70	1.53	.45	1.5	1.5	.96	.54
Niacin B3 (mg)	19.67	18.21	.58	16.4	16.0	.81	.71
Pyridoxine B6 (mg)	1.47	1.38	.38	1.3	1.3	.66	.35
Folate (μg)	239.1	234.1	.62	245.9	208.4	.39	.55
Iron (mg)	13.4	12.9	.62	12.3	11.7	.65	.97
Calcium (mg)	734.0	788.4	.47	775.2	848.4	.52	.86
Selenium (mg)	.04	.04	.48	.04	.04	.68	.89
Weight (lbs.)	143.80	145.5	.001	147.1	148.2	.14	.04

The Specific Role of Genistein in Estrogen Metabolism (N. Kumar)

TABLE 3. SOY PRODUCTS CONSUMPTION AT BASELINE AND END OF STUDY

	ISOFLAVONE SUPPLEMENTED (n=33)		PLACEBO (n=33)	
	Initial	**Final**	**Initial**	**Final**
Soy Isoflavones	0	12.23	0	12.50
Other Soy Products	0	0	0	0

The Specific Role of Genistein in Estrogen Metabolism (N.Kumar)

TABLE 4. SERUM STEROID HORMONE CONCENTRATIONS AT BASELINE AND END OF STUDY

	ISOFLAVONE SUPPLEMENTED (n=33)					PLACEBO (n=33)					
	Initial		Final			Initial		Final			
	$\overline{X}$	SE	$\overline{X}$	SE	P	$\overline{X}$	SE	$\overline{X}$	SE	P	P*
Free Estradiol pg/ml	1.37	.21	1.28	.19	.47	1.54	.18	1.59	.25	.19	.48
Total Estradiol pg/ml	44.58	4.31	47.26	6.66	.08	47.39	4.34	52.03	14.4	.76	.67
Estrone pg/ml	84.16	13.76	28.50	11.75	.52	98.30	13.31	100.00	16.76	.92	.58
SHBG nmol/L	44.38	3.81	41.29	4.01	.05	46.85	3.10	45.06	3.66	.44	.55

The Specific Role of Genistein in Estrogen Metabolism (N.Kumar)

TABLE 5. MENSTRUAL CYCLE LENGTH FROM BASELINE TO END OF STUDY PERIOD

	ISOFLAVONE SUPPLEMENTED (n=33)		PLACEBO (n=33)		
Cycle	$\overline{X}$	SE	$\overline{X}$	SE	P
1^{ST}	26.27	.85	27.82	.73	.17
2^{ND}	27.84	.69	27.12	.53	.41
3^{RD}	29.81	1.02	27.55	.75	.08
Cycle 3 – Cycle 1	3.52	1.35	(-) .06	1.12	.04

The Specific Role of Genistein in Estrogen Metabolism (N.Kumar)

TABLE 6. FOLLICULAR CYCLE LENGTH FROM BASELINE TO END OF STUDY PERIOD

	ISOFLAVONE SUPPLEMENTED (n=33)		PLACEBO (n=33)		
Cycle	$\overline{X}$	**SE**	$\overline{X}$	**SE**	**P**
1^{ST}	11.79	.54	13.19	.52	.06
2^{ND}	13.37	.69	13	.44	.64
3^{RD}	13.62	.8	13.07	.34	.51
Cycle 3 – Cycle1	1.46	.63	.14	.43	.08

APPENDIX E

Phase IIA Chemoprevention Study of Selenium in Persons at Risk for Lung Cancer

Jeffrey P. Krischer, Ph.D.

PHASE IIA CHEMOPREVENTION STUDY OF SELENIUM IN PERSONS AT RISK FOR LUNG CANCER

INTRODUCTION

Much knowledge has been acquired about the multi-step process of carcinogenesis in the lung during the last 20 years. Tumorigenesis appears to be the result of a number of genetic insults, although it remains to be determined whether there is a necessary sequence or a critical number of events required. Certain genetic alterations can be detected in the bronchial epithelium of persons at increased risk for lung cancer. Selenium may act through several different mechanisms of action, including stimulation of apoptosis, protection of tissue against oxidative damage, inhibition of tumor growth, reduction of mutagenic activity and reduction of activation of carcinogens and stimulation of the immune system. Selenized yeast has also recently been shown to reduce lung cancer incidence and mortality in a population of skin cancer patients. Smokers and survivors of early stage lung and head and neck cancers have had a long period of promotion by carcinogenic agents on the bronchial epithelium resulting in morphologic and molecular alterations. **We hypothesize that these morphologic and molecular alterations can be detected and modulated by chemopreventive agents.** We have proposed a Phase IIa chemoprevention trial evaluating five different dose levels of selenium administered daily for 3 months in subjects at high risk for lung cancer with bronchoscopically documented dysplasia. After establishing the maximum tolerated dose, additional subjects will be entered at that dose level in order to examine the modulation of biomarkers in response to selenium supplementation as well as to measure selenium levels and modulation of glutathione peroxidase as a measure of drug effect. In addition to morphology, the surrogate endpoint biomarkers to be examined include apoptosis, p53 expression, K-ras mutation analysis, p16 methylation, and upregulation of hnRNP A2/B1. Successful completion of this study will support selenium supplementation as potentially beneficial therapy in preventing the progression of lung carcinogenesis as well as identify surrogate endpoint markers that appear to be modulated by selenium supplementation.

BODY

The Statement of Work was submitted as a timeline. Initial patient accrual refers to the enrollment of subjects on the dose-finding portion of the study, and this is still currently underway. No secondary patient accrual has begun, as completion of the first phase is required before continuing on to the second phase.

Since October 1, 1999, 5 subjects have consented to participation and undergone sputum induction. One was eligible to proceed to bronchoscopy but did not have metaplastic or dysplastic biopsy samples. Fourteen subjects have completed the dose finding phase of the study. Screening for the last subject needed for the dose selection phase is in progress at the Lifetime Cancer Screening Center. We have opted for a limited screening of study subjects for recruitment of the last individual needed for the completion of the dose finding phase of the study.

A total of 499 subjects have been screened for the study, with 153 eligible for the study. Of those eligible, 151 chose to participate in the study. Sputum induction has been performed on 151 individuals. Forty were normal and 102 showed some abnormality, with 9 specimens showing inadequate levels of cells. Fifty-three of those 102 subjects elected to continue on to the bronchoscopy. Analysis of bronchial biopsies resulted in 14 subjects being eligible to take part in the selenium dose selection phase of the study and all agreed to participate. All fourteen subjects have completed three months of selenium at the various dose levels. Recruitment and work-up continue to place the 15th subject on study in order to complete the dose finding phase of the study.

Demographics

Of the 151 subjects who had a sputum induction, 96 were male and 55 female. The group consisted of 90 current smokers and 61 former smokers. Of the 53 subjects who proceeded with bronchoscopy, 41 were male and 12 were female. This group consisted of 23 current and 30 former smokers. Mean pack-years of smoking were 61.2 for males, 58.1 for females, 51.8 for former smokers and 72.4 for current smokers.

Sputum results

Sputum inductions on 151 subjects produced the following results:

Sputum Cytology Results	Number
No Significant Abnormality	40
Metaplasia	82
Mild Dysplasia	17
Moderate Dysplasia	2
Severe Dysplasia	1
Insufficient material/Not interpretable	9

The 102 subjects with metaplasia or dysplasia were eligible to proceed to bronchoscopy.

Bronchoscopy Results

Bronchoscopy was performed on 53 individuals. Those with dysplasia qualified to enter the selenium dose selection. Fourteen individuals were randomly assigned to one of five dose levels of selenium for 3 months and had a second bronchoscopy upon completion. The bronchoscopy results were as follows:

Bronchial Histopathology	Initial	3 Month
No Significant Abnormality	14	0
Basal Cell Hyperplasia	0	1
Regular Metaplasia	23	1
Mild Dyplasia	11	8
Moderate Dysplasia	4	3
Severe Dysplasia	1	1
CIS	0	0
Invasive Cancer	0	0
Total	53	14

Patient ID	Baseline Pathology Diagnosis	3 Month Pathology Diagnosis
4	Mild dysplasia	Mild dysplasia
10	Mild dysplasia	Mild dysplasia
14	Moderate dysplasia	Mild dysplasia
20	Mild dysplasia	Mild dysplasia
31	Mild dysplasia	Mild dysplasia
39	Mild dysplasia	Basel cell hyperplasia
48	Mild dysplasia	Moderate dysplasia
78	Mild dysplasia	Mild dysplasia
82	Mild dysplasia	Moderate dysplasia
130	Moderate dysplasia	Severe dysplasia
136	Mild dysplasia	Mild dysplasia
140	Mild dysplasia	Moderate dysplasia
145	Moderate dysplasia	Mild dysplasia
146	Moderate dysplasia	Regular metaplasia

Data is presented by smoking and bronchoscopy finding in figures 1-3.

Selenium Blood Levels

Blood samples taken from 14 selenium participants at baseline and 3 months show increase in blood selenium levels for all subjects. Average selenium level at baseline was 130 mcg/l and at 3 months was 305.5 mcg/l. The selenium levels for those who did not proceed with the study was 131.7 mcg/l.

Adverse Events

A total of eight adverse event reports were filed, none of which were determined to be study related. Two adverse event reported for study subject number 146 were thought to be an outcome of either anesthesia or dehydration from fasting for the bronchoscopy. A summary of the events is shown below.

Patient ID	Date of Event	Event	Study Related	Outcome
003	5/01/98	Asthma	No	Resolved
010	8/18/98	Cough, chest tightness, fever	No	Resolved
010	8/25/98	Follow-up	No	Resolved
074	12/17/98	Vaso-vagal response, post bronchoscopy	Possibly	Resolved
014	1/10/99	Hernia	No	Herniorrhaphy
014	2/01/99	Fever	No	Resolved
146	12/3/99 to 12/5/99	Migraine headache	Possibly	Resolved
146	8/6/99 to 8/9/99	Migraine headache	Possibly	Resolved

No selenium dependent toxicity was reported for any dose level.

Laboratory Progress Report

For the 14 individuals who have completed the study:

- 140 biopsies have been formalin fixed and paraffin embedded, with most already sectioned in preparation for use.
- 72 biopsy samples have been micro-dissected for collection of atypical and normal cells from the same site.
- 28 blood samples, with GPX assays done in triplicate including control assays for no substrate, no sample and a positive control with purified GPX.
- Methylation specific PCR (MSP) of the p16 gene has been done on both the baseline and post-selenium sputum samples. No aberrant methylation patterns have been detected.
- MSP has been done on DNA extracted from bronchial cells collected at the biopsy site but failed to attach to the culture dishes. To date, 192 samples have been tested for MSP of the p16 gene. Using control primers, 158 of the samples could be analyzed (82.3%) but only one sample showed a positive result for MSP of the p16 gene (0.52%). We plan to immunostain for p16 on biopsy samples as well.
- Assays are currently under development for the detection of K-ras mutation in codon 12.
- Assays are currently under development for the detection of overexpression of p53 protein using a fluorescent tagged antibody and a laser scanning cytometer (CompuCyte).

For all study subjects

- Tissue culture of normal bronchial epithelial cells on 53 study subjects
- 283 NHBE cultures have been attempted with 139 growing well enough for the collection of cells for the preparation of DNA or stored for regrowth.
- DNA has been prepared from either the cells grown in culture or from cells that failed to attach to the culture dish.
- 53 blood samples processed for isolation of lymphocytes and for the baseline measurement of GPX plus an additional 14 samples from individuals who completed selenium supplementation.
- All samples, whole blood and lymphocytes, are stored at -80°C. All NHBE cultures are stored in liquid nitrogen.
- All biopsy blocks are stored and controlled by the Pathology Department of the H. Lee Moffitt Cancer Center. In general, for most study subjects the number of biopsies is the same as the number of sites used for collecting NHBE for cell culture. For a few study subjects, extra biopsies were taken at the discretion of the physician.
- Comparison interpretation by a blinded consultant (Dr. Gazdar) has been completed and those interpretations have been compared with those by our study pathologist (Dr. Khoor). No significant difference in interpretation was found (Figure 4).

KEY RESEARCH ACCOMPLISHMENTS

- Established feasibility of recruiting and enrolling heavy current and former smokers on a chemoprevention study
- Developed algorithm to recruit and screen subjects, obtain induced sputum specimens, obtain history and physical and screening chest x-ray and blood work prior to bronchoscopy, obtain bronchoscopy and start eligible subjects on selenium supplement.
- Developed close collaborative relationships with pulmonary medicine and pathology
- Evaluated induced sputa from high risk individuals for p16 hypermethylation (all with no hypermethylation detected)
- Developed archive of 274 bronchial epithelial cell cultures
- Developed archive of induced sputum specimens from 151 high risk individuals
- Evaluated the value of fluorescent bronchoscopy in addition to white light bronchoscopy in predicting dysplasia in a high risk population (Figure 5).
- Measured glutathione peroxidase pre- and post-selenium supplementation and found no change
- Recent literature report had shown that the levels of a selenoprotein, thioredoxin reductase, increased when exposed to sodium selenite (1). An antibody directed at thioredoxin reductase was used in both western blot and immunohistochemical staining. In the western blot, BEAS-2B cells were treated with either selenomethionine or selenite, but only selenite showed a partial response for a band of the correct size. Unfortunately, the western blot also showed many other bands which would confound any immunohistochemical staining. We did stain slides from two biopsies taken before and after selenium supplementation from four individuals. We saw no obvious difference. We will attempt to find another antibody for thioredoxin reductase that might be useful.
- Measured selenium pre- and post-selenium supplementation and found increase (dose still blinded)

REPORTABLE OUTCOMES

- Development of repository of induced sputum specimens from 151 individuals
- Development of repository of bronchial epithelial cell cultures (274 cultures from 53 study subjects).
- Serum and lymphocyte repository from the same patient population.
- Fluorescent light bronchoscopy better predicts atypia than white light bronchoscopy (Figure 5).
- No detectable p16 hypermethylation was found in DNA samples from sputum collected at baseline for the 14 individuals who received selenium supplementation.
- NHBE cells cultured in the laboratory were tested for the presence of hypermethylation of the p16 promoter region. From a total of 192 cultures tested for hypermethylation of the p16 promoter, we found that 158 cultures could be amplified (82.3%) with only one culture positive (0.52%).

CONCLUSIONS

The dose levels of selenium used in the initial phase of the protocol appears to have no obvious toxicity but we need to finish the recruitment before we can unblind the study. We assume at least two individuals have received the highest dose of selenium (1000 μg).

Blood selenium levels increase after supplementation with selenomethionine. Baseline levels range from 92-200 μg/l and the levels after supplementation range from 190-460 μg/l. Once we unblind the study, we will be able to determine which doses are above the saturation level but we will still need to determine which dose is effective at modulation of atypia or biomarker.

Based on our current analysis, fluorescent bronchoscopy is better able to detect atypia than white light bronchoscopy. This analysis confirms the anecdotal comments we were receiving from the bronchoscopy physicians and personnel.

Analysis of the hypermethylation of the p16 promoter region has, so far, not shown any significant alteration of the region to suggest repression of transcription. We failed to detect a methylation specific PCR product in either sputum samples or in cells collected from the biopsy site. The samples we used to prepare DNA may have contained insufficient atypical cells for detection of an altered gene. We are currently immunostaining biopsy specimens for the expression of p16 protein.

Glutathione peroxidase activity is not an adequate marker for measuring different levels of selenium. None of the samples collected after selenium supplementation showed a significant change in activity which would result from increased selenium in the blood. Even though we do not know the dose level for the different study subjects, we do know the blood levels. Apparently, the normal baseline levels of selenium are sufficient for optimal enzyme activity and this assay will not be used after completion of the dose finding phase.

The lack of any reportable selenium toxicity suggests that the optimal dose level for a selenium chemoprevention trial is not able to be set at the range of doses studied. The external scientific advisor committee, after hearing a presentation on this study in November, 1999, recommended finishing the current trial for the planned study period (Phase I), but not initiating another trial (the Phase II portion) using selenomethionine unless a better rationale and a more focused approach was developed. The current activities focus on completing Phase I and address the laboratory studies originally proposed.

REFERENCES

1. Berggren, M., Gallegos, A., Gasdaska, J. and Powis, G. 1997. Cellular thioredoxin reductase activity is regulated by selenium. Anticancer Research 17:3377-3380.

APPENDICES

Figure legend and Figures 1-7.

APPENDIX

Figure Legends

Figure 1: Histopathology diagnosis correlated with smoking status. Multiple biopsies were taken for each individual. The frequency of the most severe bronchial histopathology is correlated with the smoking status obtained from the enrollment questionare.

Figure 2: Histopathology diagnosis correlated with the observed white light bronchoscopy. Multiple biopsies were taken for each individual and each biopsy has both the histopathology diagnosis as well as the interpretation by white light (White light classification is: Class I is normal; Class II is characterized by inflammation and/or metaplasia to mild atypia; Class III is characterized by moderate to severe atypia).

Figure 3: Histopathology diagnosis correlated with the observed fluorescent light bronchoscopy. Multiple biopsies were taken for each individual and each biopsy has both the histopathology diagnosis as well as the interpretation by white light (Fluorescent light classification is: Class I is normal; Class II is characterized by inflammation and/or metaplasia to mild atypia; Class III is characterized by moderate to severe atypia).

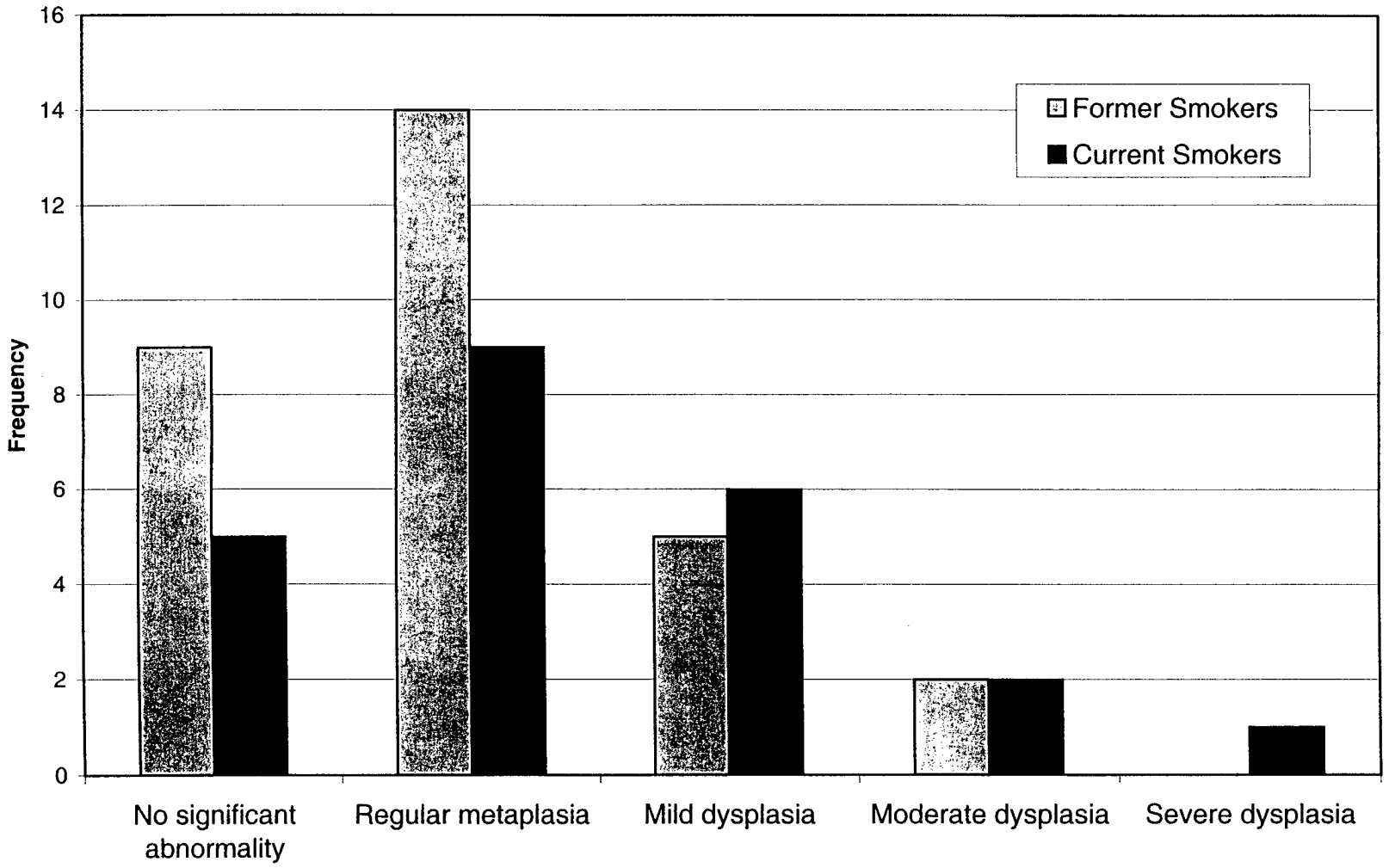
Figure 4: A consulting pathologist was recruited to validated the diagnosis of our study pathologist. The results show the percent of dysplasia diagnosed in both the biopsies (Path Findings) and in the overall diagnosis (Path Diagnosis).

Figure 5: A comparison of the atypia described during both the white light and fluorescent LIFE bronchoscopy shows that LIFE calls more severe atypia than white light. This is supported by LIFE showing a stronger correlation to the histopathological diagnosis than white light.

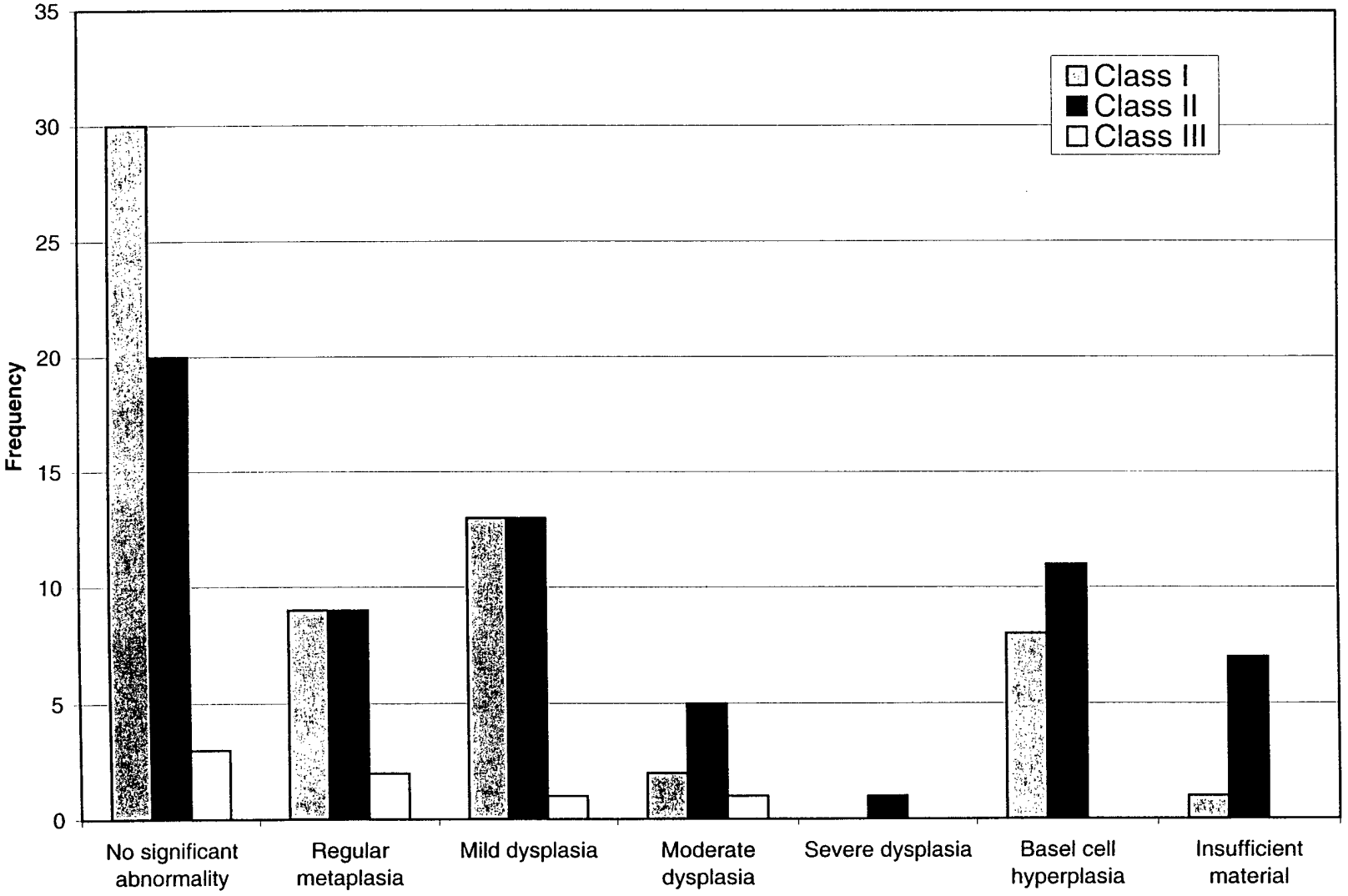
Figure 6: Hemoglobin measurements at baseline and after 3 months of selenomethionine supplementation. Total hemoglobin was measured from lysed red blood cells using a kit purchased from Sigma Diagnostics (Cat. No. 525-A). The procedure is based on the oxidation of hemoglobin to methemoglobin and the subsequent conversion of methemoglobin to cyanmethemoglobin by potassium cyanide which has a maximum adsorption at 540 nm. The color intensity measured at 540 nm is proportional to the total hemoglobin concentration. Note that hemoglobin values measured in this way are higher than those obtained with other clinical chemistries.

Figure 7: Glutathione peroxidase activity per gram hemoglobin in blood samples taken at baseline and after 3 months of selenomethionine supplementation. Glutathione peroxidase (GPX) activity assay was done in triplicate for each blood sample collected at baseline and after 3 months of selonomethionine supplementation. A separate assay with purified GPX was done as a positive control and assays without sample or the organic peroxide substrate were done as negative controls. The assay is an indirect measure of GPX activity where oxidized glutathione is produced upon reduction of organic peroxide (*tert*-butyl hydroperoxide) and is recycled to its reduced state by exogenously added glutathione reductase (GR). The reduction of oxidized glutathione by GR requires the oxidation of NADPH. The GPX activity is determined by a decrease in adsorption at 340 nm as NADPH is oxidized to NADP. Using the molar extinction coefficent for NADPH (6220 $M^{-1}cm^{-1}$) and the rate of decrease in absorbance at 340 nm, the GPX activity for each sample can be calculated.

Figure 1 **Histopathology Frequency in Current and Former Smokers**



Bronchial Histopathology Findings by White Light Bronchoscopy
Figure 2



Bronchial Histopathology Findings by Fluorescent Light Bronchoscopy
Figure 3

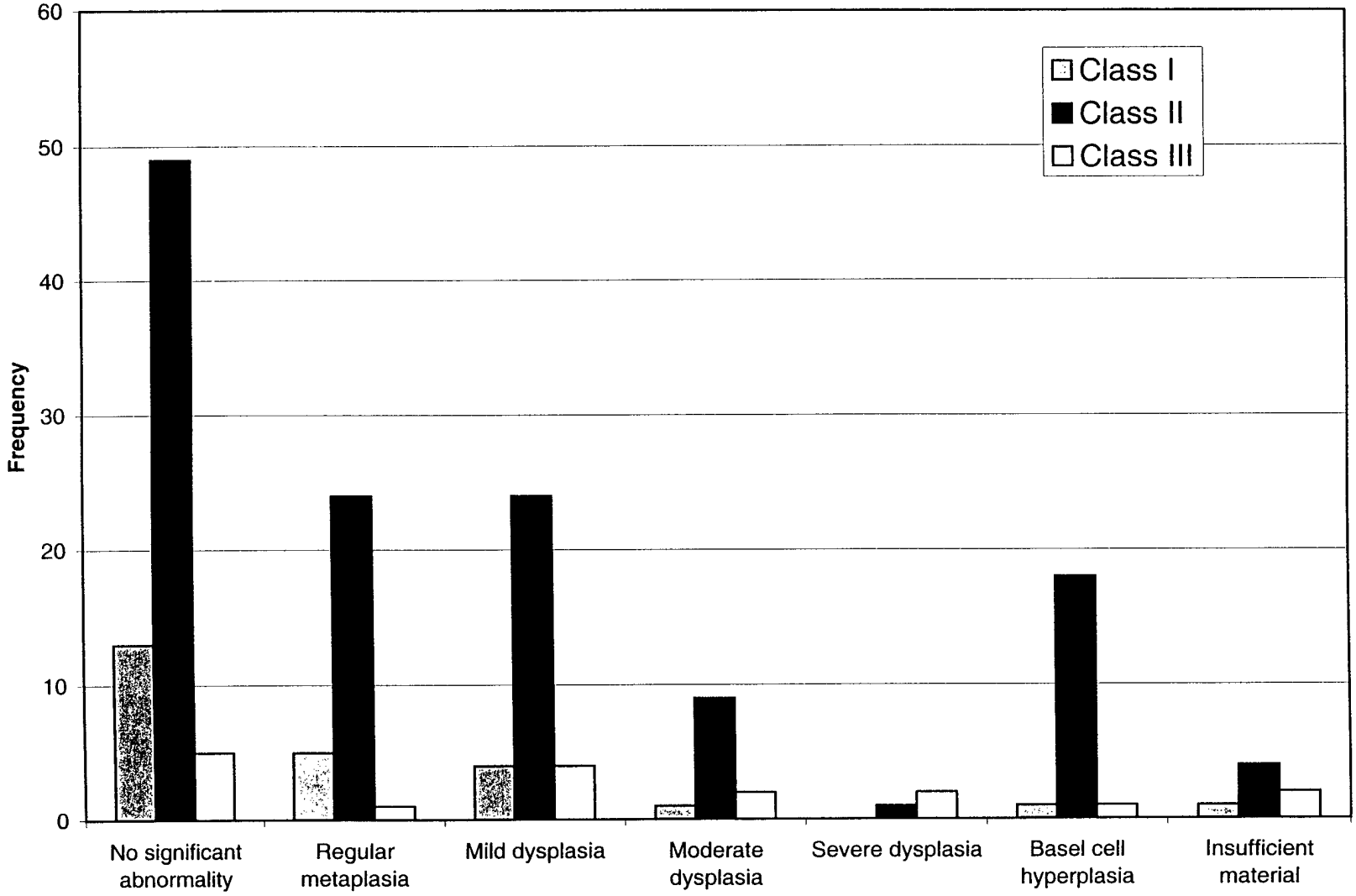


Figure 4

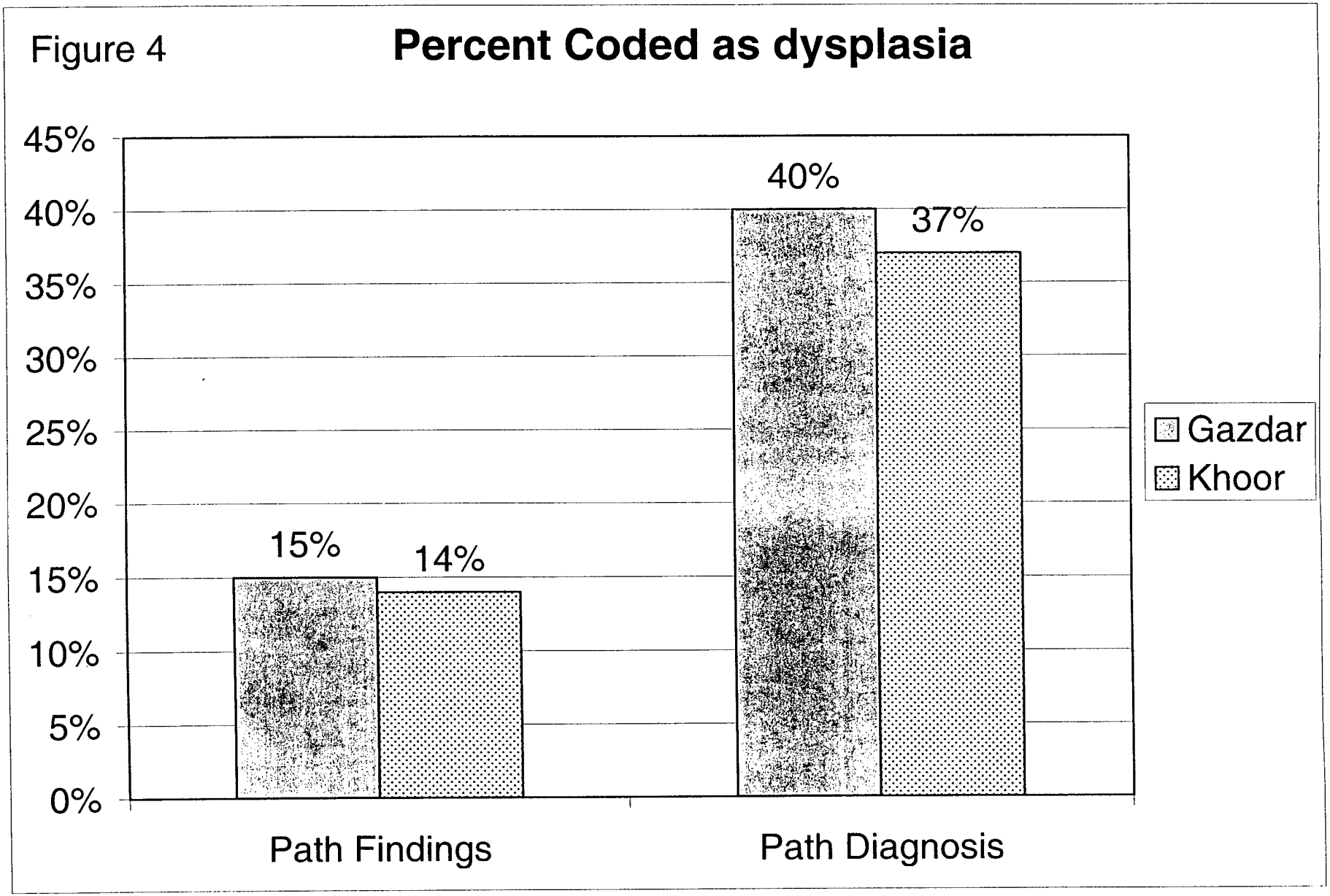

Percent Coded as dysplasia
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
15%
14%
40%
37%
Path Findings
Path Diagnosis
Gazdar
Khoor

Figure 5

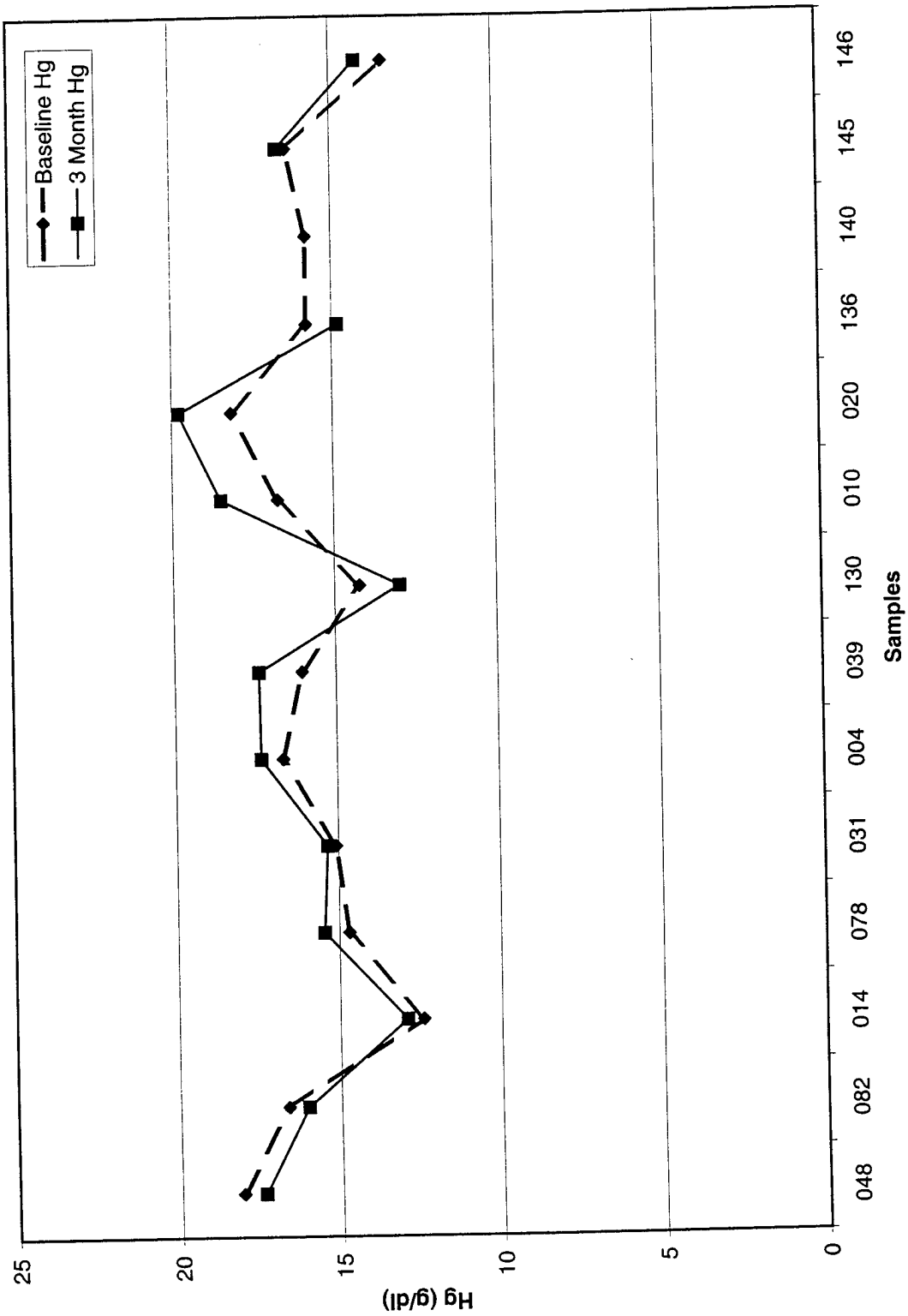
Agreement Between Observations with White Light and LIFE Bronchoscopy

		LIFE CLASS		
		I	II	III
WHITE LIGHT CLASS	I	156	81	5
	II	13	15	2
	III	2	0	2

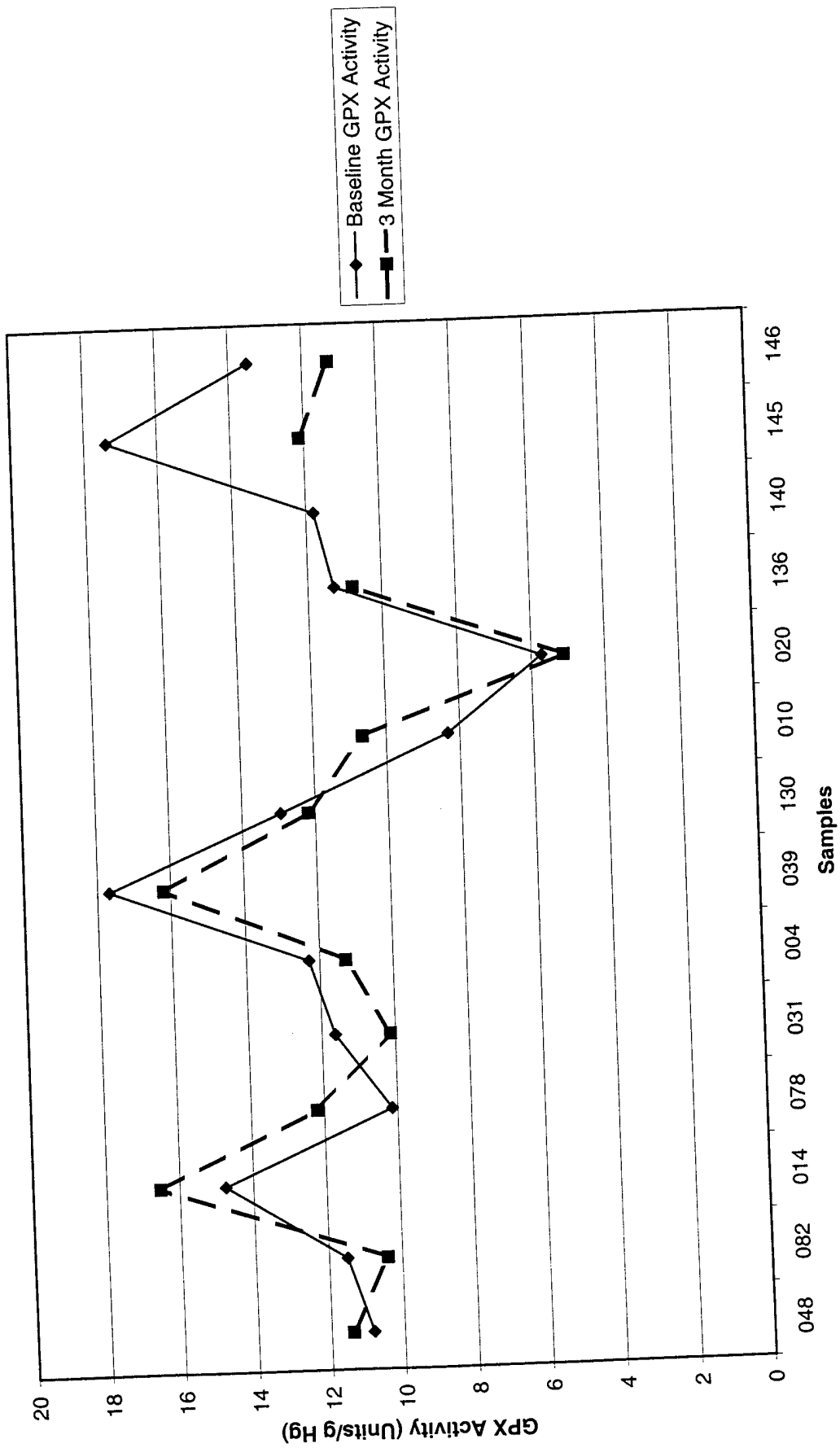
Observations by LIFE bronchoscopy of 276 endobronchial sites correlated with those by white light bronchoscopy (Spearman correlation coefficient R=0.16, p=0.0087). Agreement was not strong with Kappa statistic=0.108 (95% confidence limits 0.014, 0.20).

Histopathological diagnosis showed a better correlation with LIFE bronchoscopy (R=0.12, p=0.053) than with white light bronchoscopy (R=-0.075, p=0.233).

Hemoglobin Levels
Figure 6



Glutathione Peroxidase Activity
Figure 7



APPENDIX F

Development of Moffitt Cancer Network as a Telemedicine and Teleconferencing Educational Tool for Health Care Providers

Jeffrey P. Krischer, Ph.D.

INTRODUCTION:

The Moffitt Cancer Network's (MCN) goal is to provide up-to-date oncology related information, resources, and education to oncology health care providers and researchers for the prevention and cure of cancer. Consistent with the aims of the Advanced Cancer Detection Center, the MCN provides access to educational programming, cancer control and clinical protocols, and a mechanism to exchange patient focused information leading to the improved detection and treatment of cancer. The MCN is health care provider focused and complements an array of existing public/lay information sources available elsewhere. It is built around the concept that oncology expertise is geographically centralized, multidisciplinary in nature and of limited availability. The MCN addresses these constraints by increasing availability through a World Wide Web-based design that enables wide access from many geographic locales. The objectives of this project are to:

- Collect and organize cancer information to provide educational content to physicians and other health care providers,
- Develop and implement software to encode video and audio to enable viewing over the Internet at a range of speeds (bandwidths),
- Implement a mechanism to deliver continuing education credits through on-line testing and automated submission/evaluation,
- Design and create a web page to permit easy sorting, searching and selection of educational programming,
- Design and create a web page to deliver physician referral information that includes submission of an electronic case record consisting of text and imaging data, and
- Provide access to case conferencing from remote locations using easily available audio/video to the desktop.

BODY:

Task 1. Collect and organize cancer information to provide educational content to physicians and other health care providers. (Months 1-60).

A schedule of events is determined in coordination with the Moffitt Office of Conference Planning, the USF Department of Education, the USF department of Continuing Medical Education and independent researchers wishing to present. These events include: Grand Rounds, the monthly meeting of the Cancer Control Research Interest Group (CCRIG), a number of national and local oncology conferences, as well as, more recently added, a number of JCAHO requirements for in-service education for nurses, physicians, and other hospital staff.

The MCN currently 164 presentations in its library, increasing at a rate of 2.3 presentations per month on average. Additionally, 11conferences sponsored by USF and Moffitt are also currently available online.

Schedule videographer coverage of grand rounds and research conferences.
The Network Coordinator in cooperation with Moffitt Department of Education compiles a schedule of events. This schedule is used to determine the scheduling needs of the MCN videographer. The MCN videographer provides audio and video capture of these events on to 90 minute DVCAM (Digital Video Camera) tapes, which are then digitized by the MCN.

Coordinate notification of nursing, pharmacy and other health care providers continuing education presentations.
The Moffitt Department of Education notifies the MCN of all continuing education presentations and obtains a release from all speakers that permits the distribution of their respective presentation by the MCN.

Organize the videotaping of faculty scientific presentations for national oncology conferences.
The notification and videotaping of national oncology conferences is scheduled in accordance with the system mentioned above, developed in coordination with the MCN and the Moffitt education department. A number of conferences have been added to the MCN library. These presentations are digitized and are made available on the MCN website. The presentations acquired by this activity are codified by continuing education, searchable by subject and grouped by their respective conference title.

Coordinate with the Department of Education notification and scheduling of relevant conferences.
The Moffitt Department of Education notifies the MCN of all relevant conferences and the MCN videographer is scheduled in accordance with the videotaping needs of each conference.

Task 2. Develop and implement software to encode video and audio to enable viewing over the Internet in a range of speeds (bandwidths). (Months 1-60)

Explore the application of the Tag development software to support multiple video connections and the impact on network bandwidth.
The MCN has developed a process of digitizing presentations using the Digital Renaissance Tag Composer. Through this process MCN is able to stream presenter's slides and audio simultaneously by using a Synchronized Multimedia Integration Language (SMIL) script file. MCN originally encoded presentation for distribution over ISDN speeds of 128k and modem speeds of 56k. The encoding process used previously created two-network streaming formats, one for ISDN speed connections at 128 kilobytes per second and a second format for current modem technology speeds of 56 kilobytes per second or less. Using the Real media server software, users linking to a presentation acquire the format (streaming speed) appropriate for their connection bandwidth. The server and the user's player handle this process automatically. Late in August 2000 MCN determined that the ISDN format was redundant, as it did not offer any significant improvement over the modem format due to the low frame rate of the presentations being developed (sometimes as low as one frame for every three minutes), and MCN has discontinued the encoding an ISDN bit rate media file and thus lowering the production time.

Evaluate alternative connectivity models, including cable modem connections or access to cable networks as a means to enhance distribution of educational content.
The MCN has evaluated multiple alternative connectivity models, including cable modems, ISDN, ADSL, and traditional T1 & T3 service lines. We have found that cable modems are an excellent method of distributing educational content. Cable modems provide a low cost, high bandwidth alternative for the user. This allows educational content to become more dynamic and interactive increasing the quality and effectiveness of the educational activity.

Evaluate the Internet 2 as to its availability to sustain the necessary bandwidth for the Moffitt Cancer Network.
Moffitt Information Technology is currently evaluating Internet 2. Development in this area will depend on the more general availability of the Internet 2 to MCN users.

Resolve firewall and security issues to provide secure communication for clinical data as well as to adequately deal with subscriber/user requirements for security to permit desktop access.
A firewall has been put in place to ensure secure communications for clinical data and to address user security issues. Moffitt IT, in coordination with the MCN is currently working to develop firewall policy relating to streaming media. In August 2000, MCN moved towards streaming media as UDP packets, as opposed to only TCP packets. By doing so, caching of media streams is nearly eliminated. This required an extensive review of firewall issues. We are now looking at new processes that will embellish firewall security. Only designated ports will be available to predetermined medical professionals. In addition, data will only be available at pre-selected times and with pre-selected permission or authorization levels.

Uniform Resource Locator based on specific one-time virtual names.
All prerecorded media will be encrypted in the near future and will have unique keys for specific use. Additional security methods are still being researched and firewall security is a priority.

Expand the number of Authorized users to the Moffitt Cancer Network.
Expansion of authorized users is critical to the digital convergence with MCN's on going research and development. We are now capable of delivering "On-demand", encrypted, and live media to desktops both user specific and publicly when appropriate. In addition, with the recent addition of continuing credit hours for nursing, we have opened a huge medical audience for MCN.

Task 3. Implement a mechanism to deliver continuing education credits through on-line testing and automated submission/evaluation. (Months 1-60).

Arrange for automated notification of Department of Education staff for each new presentation selected for the Moffitt Cancer Network.
Prior to inclusion in the MCN, the Moffitt Department of Education reviews each presentation for quality of educational content.

Establish ongoing procedures to obtain releases, objectives and CME questions to implement to permit encoding of presentations and inclusion onto the Moffitt Cancer Network.
Presenters sign a release to rebroadcast prior to the videotaping of their presentation. The Moffitt Department of education works closely with the presenter and the MCN to establish objectives, determine appropriate CME questions and evaluate the overall quality of the educational content of the respective presentation. Upon the completion of this work, all information is passed to the MCN for inclusion into the MCN website for delivery to the user.

Create documentation and procedures to collect appropriate demographics on individuals desiring CME and implement electronic automated notification of our Continuing Education Office to authorize and verify CMEs earned.
Appropriate demographic information is collected from all individuals wishing to receive CME credit for physicians or nurses contact hours. Upon completion of a CME credit or contact hours, the MCN staff is electronically notified. The results of the activity are graded electronically and the information is forwarded to the USF Education Department if a CME credit or contact hour was in fact earned.

Automatically link the Cancer Library to the acquisition process so that they are aware of new acquisitions and receive opportunities to extract key words for indexing, sorting and searching.
Upon the completion of the digitization of a presentation, the digitized presentation is forwarded to the Cancer Center Librarian for review. The Cancer Center Librarian extracts key words used for indexing, sorting and searching presentations on the MCN website. These keywords are added to the MCN website database for each respective presentation.

Extend the CME process to include CEUs for nursing and pharmacy.
The MCN currently offers CME credit for physicians as well as, more recently added, contact hours for nursing continuing professional education (CEU). The certifications are provided in cooperation with the USF College of Medicine and Nursing, respectively. We are continuing to explore the applicability of the content to other healthcare providers, such as pharmacists, and the requirements to offer continuing education credits.

Expand the educational content offerings to include mandatory requirements for risk analysis, HIV, infection control, etc.
The MCN has expanded the educational offerings to include a number of JCAHO requirements for nurses, physicians and staff. These offerings are available internally to all personnel via the Moffitt Cancer Center Intranet.

Task 4. Design and create a web page to permit easy sorting, searching and selection of educational programming. (Months 1-24)

Organize educational content along primary audience lines and develop a key word searching algorithm to subset for presentations.
An algorithm has been developed allowing keyword searching. The keywords are determined during the review of the presentation by the cancer center library. A new algorithm was developed this year allowing a more efficient search. The MCN website provides chronological

ascending/descending, keyword search, search within results, and presenter last name, first name searches.

Implement a database for key words according to a standard nomenclature, utilizing NLM MeSH headings, cancer site, etc.
A keyword database has been created and is used by the MCN website for searching. The keywords are determined by the Cancer Center Librarian prior to the addition of a presentation to the MCN. The keywords are based on NLM MeSH standards.

Expand implementation of Active Server Page (ASP) extensions to the multimedia hypertext (HTML) by adding onto the 'back-end' of the Web application i.) procedural language scripting and ii.) the ability to exchange information with a fully functioning database.
ASP has been used throughout the site to produce dynamic, database driven web pages. ASP is used in all areas of the site to set procedural paths, increase security and generate dynamic content from the MCN databases.

Expand and refine the JET database to incorporate user defined search phrases that are located within a variety of fields associated with the database, including a textual 'objectives' section, MeSH headings, cancer site, canned search categories, etc.
The MCN has increased the capability of the Jet database to allow user defined search phrases. These phrases search for matches in the textual 'objectives' section, MeSh headings (keywords), cancer site, and canned search categories.

Monitor utilization by remote site to evaluate the frequency and demand for various types of educational content to permit refinements and revisions to improve offerings.
The MCN gathers extensive information in regards to use of the MCN website. This information includes website traffic, time spent, the number of presentations watched, for credit or not, and the frequency each presentation is watched.

Task 5. Design and create a web page to deliver physician referral information that includes submission electronic case record consisting of text and imaging data. (Months 1-36)

Develop and implement a database to archive text and imaging data for retrieval by consulting Cancer Center physicians and integration with Moffitt Cancer Center clinical information systems.
MCN plans on migrating its current database to a Microsoft Structured Query Language database before the end of the year. Since its media is stored as objects now, its future database will be based on usage of objects. As of July 2000, MCN began storing media and text, the former in two object formats based on Real and Microsoft Media.

Develop a structured computerized clinical case description that provides a minimally relevant set of data that describes a clinical case for second opinion and consultation.
Efforts to date have focused on image transfers and the capability to be DICOM compliant. Appropriate mechanisms have been developed along with interfaces to hospital PACS and

Radiology Departments. Exploration is currently underway to exchange textual information and establish the computerized clinical case record.

Acquire hardware and software to provide audio and video real time and time shifted streaming of case conferencing to remote locations for user viewing over secure communication links.
In July 2000 MCN procured rack mounted dual processor servers and audio/video equipment for the purpose of providing both real-time streaming of media as well as simultaneous capture of that media for archive.

Establish the necessary gateways and bridges to provide connections at a range of bandwidths to support remote connectivity.
See, also Task 2. All processes will be controlled remotely and is designed for live to archive times of no more than 5 minutes. In other words, five minutes after a live broadcast event is completed, an "On-Demand" rebroadcast will be available to specific users. The former being broadcast via secure port and virtual link and the latter are encrypted for use with a specific key. Software development for all new processes is scheduled to begin in the first quarter of 2001.

Design and implement web-based front ends to Moffitt Cancer Center clinical systems to permit secure access to patient information of patient's referred or submitted to case conferencing or second opinions.
MCN and Moffitt have collaborated to create a total package for streaming media distribution. Internally, Moffitt is hardware ready to multicast media events and with the establishment of a new dedicated media server in August 2000; it has implemented a load-balanced high bandwidth portal for streaming media for both the Intranet and Internet. In addition, using specified unicast stations; MCN can deliver media events to other facilities that can multicast and therefore reducing the bandwidth load on MCN's media server. A test of the Unicast/Multicast processes is currently being planned.

Task 6. Provide access to case conferencing from remote locations using easily available audio/video to the desktop. (Months 1-48)

Complete telegenetics experiment to assess feasibility and acceptability of this format for the exchange of clinical information.
Telemedicine began to accrue subjects on May 11, 2000. To date, 15 individuals have been asked to participate in the study, and 11 were enrolled. Based on these numbers the participation rate in the study is 73%.

Of those who refused to participate:

- none had any prior experience with video conferencing
- Three of the four had used computer several or many times and only had used a computer only once or twice.

Of those who participated:

- 9 were non-Hispanic white, one was African American and one was Hispanic.

- 3 out of eleven had had some kind of video conferencing before, which was related to their work.
- 3 out of eleven had been a part of some kind of research.
- 6 out of eleven were randomized to Telemedicine counseling, while 5 out of eleven had face to face counseling
- The overall satisfaction level was very satisfactory for six out of those who were in the Telemedicine counseling arm (6)

The overall satisfaction level was very satisfactory for four and satisfactory for one out of those who were in the Face to Face counseling arm (5)

Implement additional sites to expand this program and resolve billing issues within the context of existing laws are regulations regarding telehealth and teleconsultation programs.
MCN has begun building the administrative infrastructure to deal with access, billing and scheduling issues. This includes a point of contact to receive requests, coordination of schedules for presentations and an administrative web-based front end to view a calendar of presentations and establish the appropriate login and passwords for approved permissions. New legislation is expected during the current legislative session that will have a direct bearing on regulations governing telemedicine and telehomecare.

Establish the necessary gateways and bridges to provide connections at a range of bandwidths to support remote connectivity.
See, also Task 2. All processes will be controlled remotely and is designed for live to archive times of no more than 5 minutes. In other words, five minutes after a live broadcast event is completed, an "On-Demand" rebroadcast will be available to specific users. The former being broadcast via secure port and virtual link and the latter are encrypted for use with a specific key. Software development for all new processes is scheduled to begin in the first quarter of 2001.

Develop tunneling or other secure links to resolve firewall issues regarding LAN configurations at both the Moffitt Cancer Center and remote sites.
Moffitt is using Virtual Private Networks now.

Acquire and install technology in conference centers where case conferencing generally occurs for selected clinics to permit retrieval and display of multiple images and clinical data submitted for this purpose by remote users.
All hardware has been purchased for this project and a formal walkthrough and equipment installations should begin before the end of October 2000. For each possible site, a detailed plan of operations has been developed to establish the capability to schedule and transmit signals for MCN distribution. MCN has purchased and will implement within the next few weeks streaming equipment for Pathology. This system will include audio capture and image capture from microscope slides and x-rays as well as the audience during discussions.

Assess utilization of this technology to refine and revise formats and improve the quality and ease of remote access.
As noted previously, MCN has made it a priority to improve the quality of its products. Moving towards the use of Microsoft products and its MPEG-4 streaming format will reduce labor and

increase quality across the board. MCN will be writing new programs for remote control of streaming servers and changing its current database into an SQL based database, which will improve speed and increase capacity. Finally, changes in its business practices will reduce labor and increase its quality and functionality as well as increase its customer base.

KEY RESEARCH ACCOMPLISHMENTS:

- The Moffitt Cancer Network is available to users and can be found at
  http://network.moffitt.usf.edu
- The MCN currently has 164 presentations in its library, increasing at a rate of 2.3 presentations per month on average. Additionally, 11 conferences sponsored by USF and Moffitt are also currently available online.
- All approved Grand Rounds presentations have been taped by the Moffitt Multimedia Education Resources Center (MERC) for over one year preceding this report. The video is captured on digital DVCAM 94 minute tapes.
- Since many of the presenters use only 35mm slide for their presentations, a process of creating final production audio/video Real media for streaming via TCP/IP has been developed. This process requires post-production labor and requires the best of the video's individual frames to be captured a second time to recreate higher quality computer images. MCN has made significant progress in this area and as of June 2000 has begun using presenter's PowerPoint files when ever possible to bypass the second image rendering process. This has reduced labor time from 3.5 days to about 5 hours, while increasing image quality noticeably. This labor savings is not realized when presenters are using 35mm film only.
- In addition to pre-presentation file acquisition, MCN has begun the development of a presenter packet. When finished, this packet will inform presenters to repeat important questions asked at the end of events like Grand Rounds and these will be added to the content to be available to medical professionals at the MCN website.
- National oncology conferences have been taped and included in the MCN website database. Conferences have been subdivided into their respective presentations and are categorized searchable as well as searchable using the website database Access Jet engine. All conferences are pre-qualified for their ability to become online educational materials by the University of South Florida College of Medicine and, more recently, the University of South Florida College of Nursing.
- MCN is now beginning to test and research a second media streaming process using MPEG-4. Not standardized by the World Wide Web Consortium yet, the newly introduced streaming format allows for embedded script and control processes within the media stream.

REPORTABLE OUTCOMES:

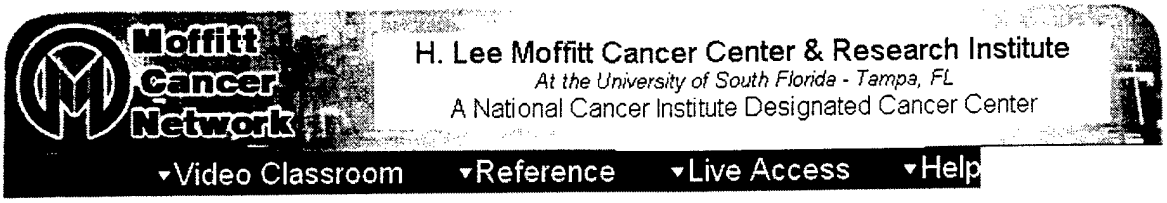
- patents and licenses applied for and/or issued;
  A notice of disclosure has been filed with the USF office of patents in anticipation of the completion of a patent application.

CONCLUSIONS:

The purpose of this research is to create processes that allow medical professional to extend their abilities through the use of electronic media. MCN has evolved in pace with the change of that technology and because of its foresight and its dedication to purpose it has kept ahead of the technology. MCN has realized that streaming media processes are not yet capable of high definition presentations at low bandwidth and has developed the best possible processes for producing usable educational media delivery using network technology. MCN's research into these processes has revealed the need for specific products and their uses. Several new programs will be developed to address these. For example, to cut down on the need for many new employees, MCN will be developing a broadcast program that will allow a single user to set start/stop times on a given event at a given location. In addition, this program must have a simple user interface that a cameraman will be familiar with, similar to a tape recorder.
Further investigation into security processes must be addressed when MCN implements streaming from doctor to doctor in case reviews including new HIPAA requirements for medical privacy and confidentiality. Providing second opinion and expert information to referring physicians is an extremely important addition to MCN's research. While continuing education is a given, in the final analysis, it may be in the medical professional interaction that MCN becomes most useful.

REFERENCES: None

APPENDICES:



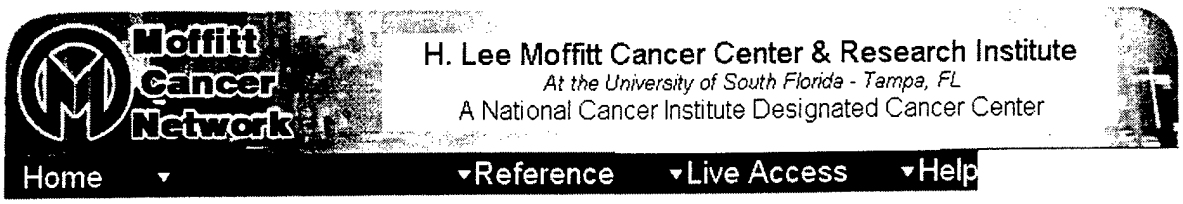
Scientific Presentations
Conferences
User History

Moffitt Cancer Network

Click on the Video Classroom for scientific presentations on cancer treatment, biology, screening and prevention research. These full-length videos are searchable by topic as individual presentations or as part of a scientific conference. Continuing education credit for physicans (CME) or contact hours for nursing education are available online at a nominal cost.

Click on Reference for an online review of alternative and complementary medicines, their intended use (i.e., claims for cancer prevention or treatment), applicable research studies, safety/toxicity and recommendations.

Click on LiveAccess for real-time scheduled scientific presentations brought to you by the H. Lee Moffitt Cancer Center and Research Institute. Special live presentations or access to case conferencing can be prearranged by contacting MCN support.



Scientific Presentations - *Sorted by Most Recent*

Date: 7/21/00
Metastatic Melanoma Model
Speaker: Steven J. O'Day M.D.
John Wayne Cancer Institute
Approved for 1 hour of CME Credit
Approved for 1 contact hour for nurses

Date: 6/23/00
New Advances in Cutaneous T-Cell Lymphoma
Speaker: Francine M. Foss, M.D.
Tufts New England Medical Center

Date: 6/16/00
The Role of STAT Proteins in Growth Control
Speaker: Jacqueline Bromberg, M.D., Ph.D.
Memorial Sloan Kettering Cancer Center
Approved for 1 hour of CME Credit
Approved for 1 contact hour for nurses

Date: 6/1/00
Stat Activation in Prostate Cancer
Speaker: Linda Mora-Diaz M.D.
H. Lee Moffitt Cancer Center & Research Institute

Date: 5/12/00
Vitamin D and Breast Cancer
Speaker: Rajendra G. Mehta Ph.D.
University of Illinois
Approved for 1 hour of CME Credit
Approved for 1 contact hour for nurses

Date: 4/28/00
Recent Advances in the Management of Head & Neck Cancer
Speaker: Dong M. Shin, M.D., F.A.C.P.
M. D. Anderson Cancer

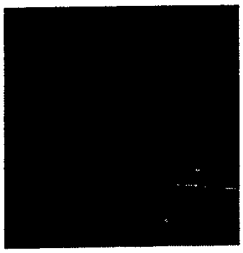
Sort Order:
Most Recent GO

Search For:
GO

Information about Continuing Education Credits for Physicians (CME)

Information about Continuing Education Contact Hours for Nurses

The Role of STAT Proteins in Growth Control



Presenter: Jacqueline Bromberg, M.D., Ph.D.
Assistant Professor
Memorial Sloan Kettering Cancer Center and the Rockefeller University
New York, NY
Date: June 16, 2000
Video Length: 55 minutes

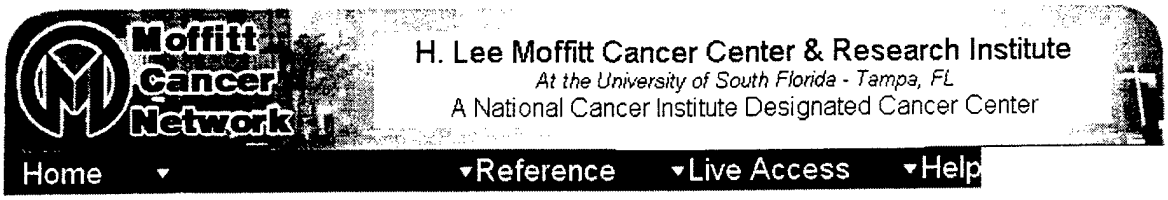
Objectives: After viewing this presentation, you will be able to:

- Discuss the importance of phosphorylation cascades in transcriptional regulation.
- Examine the critical role STAT proteins may plan in the development of cancer.

You may watch this presentation and get CME credit or 1 contact hour (when applicable) for a nominal fee of $25.00

Target Audience:
This program is intended for pysicians and nurses who are primarily involved in cancer care, research and education and is intended to advance clinical and research reasoning to contribute to the mission and prevention and cure of cancer.

This presentation requires RealPlayer.



Conferences

Click on a conference title to choose from available presentations.

End of Life - Issues in the 21st Century
April 2000

Florida Pituitary Symposium - Y2K Update - Focusing on Comprehensive Care of the Pituitary Patient
April 2000

Current Advances in Cancer Care
February 2000

Advances in Pediatric Hematology/Oncology
January 2000

Evidence-Based Practice of Oncology
November 1999

Cancer Communications - Talk, Tools & Technology
September 1999

Innovations in Breast Cancer Therapy
December 1998

End of Life Care in the 21st Century - Incorporating Palliative Care into Mainstream Medicine
November 1998

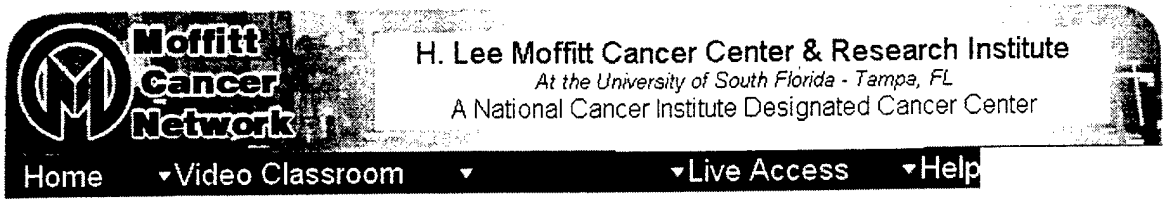
Cancer, Culture and Literacy
May 1998

Cancer Genetics for the Clinician
March 1998

Search Conferences For:
GO

Information about Continuing Education Credits for Physicians (CME)

Information about Continuing Education Contact Hours for Nurses

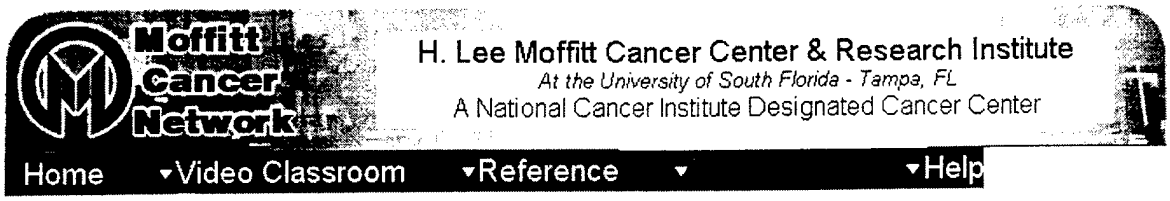


Alternative Medicine

Alternative and Complementary/Integrative Nutritional Therapies in Cancer Prevention and Treatment" by Nagi Kumar, Ph.D, R.D. This is an online review of alternative and complementary medicines, their intended use (i.e., claims for cancer prevention or treatment), applicable research studies, safety/toxicity and recommendations.

Alternative and Complementary/Integrative Nutritional Therapies in Cancer Prevention and Treatment

by Nagi Kumar, Ph.D, R.D.



Moffitt Cancer Network >

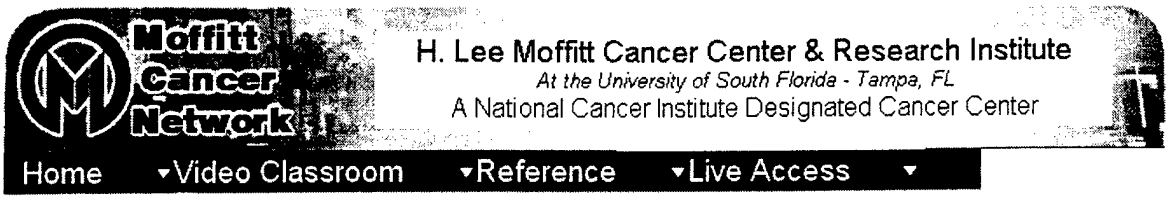
Live

About Moffitt Live Streams

Live streaming video is provided for scheduled events. These are selected from Grand Rounds and other scientific presentations at the Moffitt Cancer Center. Special services such as case conferences are available by prearrangement. Contact us for further information.

About Live Streams:

Moffitt Cancer Network live video streams require the Microsoft Media Player. To download the most current version of the Media player for free **click here.**



Moffitt Cancer Network > Help >

About Online Continuing Medical Education (CME)

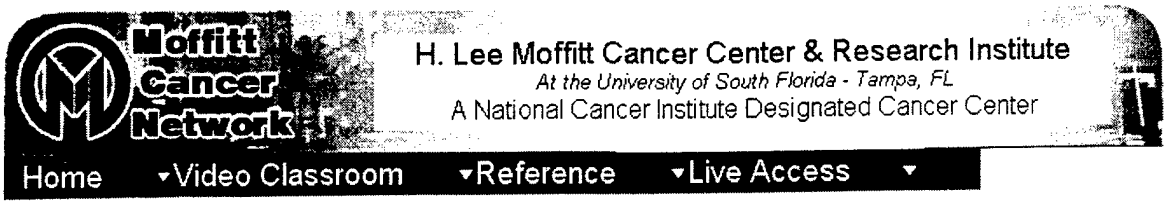
Accreditation and Credit Designation

This activity has been planned and implemented in accordance with the Essentials and Standards of Accreditation Council for Continuing Medical Education through the joint sponsorship of the University of South Florida College of Medicine and the H. Lee Moffitt Cancer Center and Research Institute. The University of South Florida College of Medicine is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The University of South Florida College of Medicine designates this educational activity for up to 1.0 hour in category 1 credit towards the AMA Physician's Recognition Award. Each physician should claim only those hours he or she actually spent in the educational activity.

Disclosure Policy:

As a sponsor accredited by the Accreditation Council for Continuing Medical Education, the University of South Florida College of Medicine must insure balance, independence, objectivity and scientific rigor in all its individually sponsored or jointly sponsored educational activities. Faculty participating in a sponsored activity are expected to disclose to the activity audience any significant financial interest or other relationship (1) with the manufacturer(s) of any commercial product(s) and (2) with any commercial supporters of the activity. (Significant financial interest or other relationship may include such things as grants or research support, employee, consultant, major stock holder, member of speaker's bureau, etc.). The intent of this disclosure is not to prevent a speaker with a significant financial or other relationship from making a presentation, but rather to provide listeners with information on which they can make their own judgments. It remains for the audience to determine whether the speaker's interests or relationships may influence the presentation with regard to exposition or conclusion.

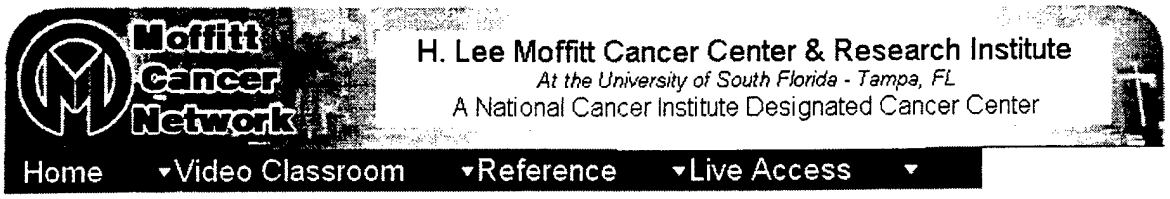


Moffitt Cancer Network >

About Online Continuing Nursing Educational Contact Hours

Accreditation and Credit Designation

This activity for 1 contact hour is provided by the University of South Florida College of Nursing, which is accredited as a provider of continuing education in nursing by the American Nurses Credentialing Center's Commission on Accreditation. Each nurse should claim only those hours he or she actually spent in the educational activity.



Moffitt Cancer Network > Help >

Sitemap

- Home
- Video Classroom
  - About Contact Hours for Nurses
  - About Continuing Medical Education for Physicians
  - Scientific Presentations
    - Sort
    - Search
  - Conferences
  - User History
- Reference
  - Alternative Medicine
- Live
  - About Moffitt Live Streams
  - Connect to Live Stream
- Help
  - Sitemap
  - Privacy Policy
  - Terms of Use

APPENDIX G

African American Families with Inherited Breast or Ovarian Cancer

Rebecca Sutphen, M.D.

African-American Families with Inherited Breast or Ovarian Cancer
Principal Investigator: Rebecca Sutphen, M.D.

1. Introduction

Although the BRCA1 and BRCA2 genes are believed to account for the majority of inherited breast and ovarian cancer, little is known about the role of these genes among African-American cancer patients, since the majority of studies of BRCA1 and BRCA2 have been performed in Caucasian families. Similarly, little data are available regarding perception of risk and attitudes about genetic testing among African-Americans. This is a study of African-American women with 1) breast cancer diagnosed at a young age and/or 2) a family history of breast and/or ovarian cancer. All participants will receive pre- and post-test genetic counseling by a geneticist or genetic counselor. Complete sequencing of the BRCA1 and BRCA2 genes will be performed and patients will have the option to receive results. The objectives of the study are to estimate the incidence of BRCA1/BRCA2 mutations in a sample of African American women compared to the incidence of these mutations in Caucasian women as predicted by various models; to examine the histologic characteristics of tumors and risk factors associated with cancer in a sample of African American patients in light of what is known about risk factors in Caucasian patients; and to explore changes in participants' cancer beliefs and attitudes during the course of genetic counseling and testing.

2. Body

The study has met all requirements of the Surgeon General's Human Subjects Research Review Board and final approval to open the study for enrollment was obtained in October, 2000. Status of tasks included in the approved statement of work are as follows:

Task 1: Subject Identification

HLMCC cancer registry data was reviewed and identified 51 living African-American breast and ovarian cancer patients who are eligible for the study, based on personal history of breast or ovarian cancer at age 45 or younger, and an additional 18 women who may be eligible for the study based on reported family history of breast/ovarian cancer.

Task 2: Subject Contact and Enrollment

Beginning in November, 2000, these patients are being invited to participate in the study either directly, at the time of follow-up appointment with their HLMCC physician or, for those patients without scheduled follow-ups, through a letter from their physician and the PI, followed by telephone contact from one of the African-American co-investigators.

Tasks 3-5 involve procedures at the time of and after enrollment.

Task 6: Data Entry and Management

Data collection instruments have been developed for this study based on validated instruments. The study will utilize the database system designed for genetic studies which is currently in use at HLMCC for other clinical genetics studies.

Tasks 7-9 involve interim analyses and reports which will be prepared.

3. Key Research Accomplishments
4. Reportable Outcomes
5. Conclusions

This study will provide important data regarding the role of the BRCA1 and BRCA2 genes in hereditary susceptibility to breast and ovarian cancer in African American families, about which little is known. It will also provide data regarding perceptions of risk and attitudes about genetic testing among African American patients. These data are essential for the development of appropriate risk assessment models for use in African American families, and toward the development of appropriate educational and counseling approaches in this group.

APPENDIX H

Molecular Fingerprint of STAT3 Regulated Genes for Early Detection of Human Cancer

Richard Jove, Ph.D.

Molecular Fingerprint of STAT3 Regulated Genes for Early Detection of Human Cancer

PI: Richard Jove, Ph.D.
co-PI: Emmanuel Lazaridis, Ph.D.

Introduction:

We originally proposed that a STAT3 molecular fingerprint may be detectable at the initial stages of cellular transformation, and thus would provide a novel molecular marker for early detection of human tumors that harbor activated STAT3. In addition to detecting the presence of tumors, the STAT3 molecular fingerprint may identify the subset of tumors with more potential for malignancy and resistance to therapy. To determine the STAT3 molecular profile associated with oncogenesis, we are using a combination of cutting-edge microarray technology and advanced bioinformatics methodology in highly collaborative studies. The ultimate goal of the present studies is to define a set of STAT3-regulated genes associated with oncogenesis that will comprise an "OncoStatChip" suitable for routine microarray analysis of tumors in the future.

Body:

Significant progress has been made towards our goal of identifying STAT3 regulated genes since funding began on July 1, 2000, for this project. We have characterized various oncogenically transformed cell lines from rat, mouse, and human origin that display elevated levels of STAT3 activation. Mouse and rat fibroblasts stably transformed with the viral oncoprotein v-Src, a potent activator of STAT3, were utilized to establish the initial screen of potential STAT3 regulated genes in oncogenesis. Total RNA was harvested from mouse fibroblast cell lines, NIH 3T3 and Balb/c 3T3 and rat 3Y1 fibroblasts stably transformed with v-Src, and prepared for analysis using the Affymetrix GeneChip Technology. The Affymetrix technology allowed us to examine the expression of 11,000 mouse genes represented on two separate chips in addition to 7,000 rat genes represented on a single chip. Control samples consisting of normal non-transformed fibroblasts not harboring the v-Src oncoprotein were also screened for gene expression using the Affymetrix technology. The data generated from the v-Src transformed fibroblasts were then compared to their normal counterparts, and genes that showed altered expression greater than 2-fold were identified for further analysis. Genes which displayed altered expression resulting from v-Src expression numbered 652 in the NIH 3T3 cells, 1568 in Balb/c 3T3, and 1045 for 3Y1 cells. Further analysis refined the list to 295 genes that were regulated by v-Src in a similar manner in both NIH 3T3 and Balb/c 3T3 fibroblasts. There was also some overlap between the rat and mouse cell lines; however, this comparison is substantially more complicated because accession numbers are unique for the same gene depending on the species. This subset of genes represents candidate Stat3-regulated genes involved in oncogenesis.

Studies investigating human STAT3-mediated gene regulation have focused on breast epithelial carcinoma cell lines. We have characterized 4 human cell lines, 2 that display elevated STAT3 activity (MDA-MB-231, MDA-MB-435), and 2 that do not

(MDA-MB-361, MDA-MB-453). Total RNA was harvested from each of the cell lines and gene expression was measured using human Affymetrix chips representing 5,600 human genes. Comparison between the STAT3-positive and STAT3-negative cell lines has identified 290 genes that show altered regulation greater than 2-fold. To further refine our list of potential STAT3 regulated genes, we treated the 2 STAT3-positive cell lines with AG490 and PD180970, two pharmacologic inhibitors of STAT3 signaling. Gene expression data indicate that AG490 treatment resulted in altered expression of 113 unique genes, and PD180970 treatment resulted in altered expression of 205 unique genes. Significantly, overlap between these two data sets identified 64 genes that displayed similar regulation following treatment by either inhibitor. These 64 genes are prime candidates for STAT3-regulated genes associated with human breast cancer.

Advanced statistical analysis of our data is currently in progress. The Affymetrix technology is considerably more sophisticated than data generated by cDNA spotted arrays. Each gene represented in the Affymetrix microarray corresponds to a calculated value from 20 unique probe sets. Based on this complexity, we have had to develop novel approaches to re-evaluate the procedure for interpreting data. We are currently designing an interface that will allow multiple statistical analyses using the raw data generated from each of the unique probe sets for each gene. Furthermore, we are developing methods to compare gene expression data across species to better define the STAT3 molecular fingerprint associated with oncogenesis. These design elements will be extremely valuable in refining our list of genes that are STAT3 regulated in breast cancer.

Key Research Accomplishments:

- Identified 295 unique mouse genes which display altered regulation by v-Src in the context of STAT3 activation and oncogenesis.
- Identified 1045 unique rat genes which display altered regulation by v-Src associated with STAT3 activation during oncogenesis.
- Identified 227 human genes that display altered expression associated with STAT3 activation in breast cancer.
- Identified 64 unique genes that display altered expression resulting from inhibition of STAT3 signaling and thus represent potential STAT3-regulated genes involved in malignant progression of breast cancer.

Reportable Outcomes:
An abstract has been submitted to the Oncogenomics meeting to be held in Tucson, Arizona, in January of 2001. This meeting is sponsored by the American Association for Cancer Research and Nature Genetics, and will be one of the major international

meetings on the application of microarray gene expression profiling to cancer. The abstract, which attached as an appendix, will be presented by Dr. Dominic Sinibaldi, who is a postdoctoral fellow bridging the research groups of Dr. Jove and Dr. Lazaridis on this project.

Conclusions:

We have analyzed various cell lines from multiple species for altered gene regulation associated with STAT3 activation in oncogenesis using Affymetrix microarray technology. Data have been generated from 18 different microarray chips. Comparison of these data has resulted in the identification of a subset of genes that are regulated by STAT3 in the context of v-Src oncogenesis and human breast cancer. Further analysis of cell lines with manipulated STAT3 signaling status is in progress to further refine our list of genes, which will ultimately comprise a STAT3 molecular fingerprint for breast cancer. Following the establishment of a STAT3 fingerprint using model cell lines, human primary breast tumors with varying degrees of STAT3 signaling will be analyzed using microarray technology and compared to the cell line expression profiles. Comparisons among all of the STAT3-associated gene expression profiles will define the subset of genes that will be evaluated as molecular markers for early detection of breast cancer. Ultimately, the STAT3 molecular fingerprint will provide valuable information for improved diagnostics and treatment of breast cancer.

Appendices:

Abstract summarizing the progress on this project submitted to the Oncogenomics conference, to be held in Tucson, Arizona, in January of 2001. This meeting is sponsored by the American Association for Cancer Research and Nature Genetics, and will be one of the major international meetings on the topic of this ACDC project.

APPENDIX I

Tampa Bay Ovarian Cancer Study

Rebecca Sutphen, M.D.

Tampa Bay Ovarian Cancer Study
Principal Investigator: Rebecca Sutphen, M.D.

1. Introduction

The BRCA1 and BRCA2 genes are believed to account for the majority of inherited ovarian cancer, yet few population-based studies have been performed specifically to investigate their role in this deadly disease. The Tampa Bay Ovarian Cancer Study (TBOCS) is a case-case study of incident epithelial ovarian cancer in the geographic region of Hillsborough and Pinellas counties, Florida, comprising an estimated 170 annual cases. A rapid ascertainment system will be utilized. In-person interviews will be conducted with all subjects, in order to collect comprehensive data on health behaviors, risk factors, personal and family history; provide genetic counseling; and obtain blood samples. Complete sequencing of the BRCA1 and BRCA2 coding regions will be performed to allow assignment of cases (mutation-carriers) and controls (non-carriers); we will thereby determine the prevalence of BRCA1 and BRCA2 germline mutations in this population. The purpose of this initial project is to demonstrate the feasibility of conducting a 5-year successor study of ovarian cancer cases with and without germline BRCA1/BRCA2 mutations. The specific aims of this feasibility study and the expanded successor study of incident epithelial ovarian cancer are: to investigate whether and which health behaviors and risk factors differ between germline mutation-associated cases and non-mutation controls; to examine differences in the family cancer history profile of mutation-associated cases and non-mutation controls; to examine differences in tumor characteristics between mutation-associated cases and non-mutation controls; and to investigate differences in response to treatment and survival between mutation-associated cases and non-mutation controls.

2. Body

Status of tasks included in the approved statement of work are as follows:

Task 1: Preparation for Medical Record Abstractions

Data elements of the medical record abstraction form have been finalized. Design of the medical record abstraction form and the data entry mechanism for pathology data has been completed. This design will allow direct data entry of abstracted medical records into the database, followed by independent review of the medical record data by the pathologist at the time of pathology analysis. This mechanism makes data entry highly efficient while ensuring data quality.

Task 2: Recruitment and Training of Study Personnel

Study staff have visited all of the recruiting physicians' offices to explain the study, review introductory study brochure and facilitate appointments for study interviews.

Tasks 3-9 involve recruitment of subjects, data collection, subject follow-up, statistical analyses and report writing. Opening the study for enrollment awaits final approval from the DOD. The study was reviewed by the Surgeon General's Human Subjects Research Review Board (SGHSRRB) on September 27, 2000. Once SGHSRRB

recommendations for revision have been received, revisions will be completed and approved by the University of South Florida IRB and forwarded to the DOD for final approval. We anticipate final approval to open the study for enrollment by November, 2000.

3. Key Research Accomplishments

Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study, funding was requested and awarded from the American Cancer Society (National) for a companion study to the Tampa Bay Ovarian Cancer Study. The 3-year companion study will evaluate biologically active plasma lysophospholipids and serum CA-125 levels of study subjects (women with newly diagnosed epithelial ovarian cancer), and healthy control women, in order to: determine the correlation between plasma lysophospholipid levels and epithelial ovarian cancer stage; compare plasma lysophospholipid levels between healthy controls and ovarian cancer patients; perform statistical analyses to determine the best correlation with ovarian cancer stage between plasma lysophospholipid levels alone, serum CA125 levels alone or lysophospholipid/CA125 levels in combination; determine the correlation between time-based plasma lysophospholipid levels and disease-free survival; and assess time-based lysophospholipid measurements as a biomarker by mathematical modeling.

4. Reportable Outcomes

Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study supported by this award, funding was awarded by the American Cancer Society for a 3-year companion study to evaluate the role of biologically active lysophospholipids for their potential as biomarkers of ovarian cancer.

5. Conclusions

Epithelial ovarian cancer results in the death of more American women than all other gynecologic cancers combined. The Tampa Bay Ovarian Cancer Study employs a unique epidemiologic design to evaluate the role of the BRCA1 and BRCA2 genes in the etiology, pathology and response to treatment of this deadly disease. Current funding is limited to support of a feasibility analysis toward a 5-year successor study. Based on the design of the feasibility analysis and successor study, a 3-year companion study has been funded to assess the potential of biologically active lysophospholipids as biomarkers in ovarian cancer. The Tampa Bay Ovarian Cancer Study and its companion study represent an important opportunity to evaluate the role of inherited susceptibility to ovarian cancer and evaluate lysophospholipids for their potential as biomarkers of this deadly disease. No similar study has been performed. In order to accomplish these critical goals, funding for the 5-year successor study will be required.

APPENDIX J

Molecular Predictors of Disease Behavior in Thyroid Cancer

Carlos A. Muro-Cacho, M.D., Ph.D.

PROGRESS REPORT

Project: **Molecular Predictors of Disease Behavior in Thyroid Cancer**

Primary Investigator: **CARLOS A. MURO-CACHO, MD, PhD**

INTRODUCTION

Every year, 18,000 new thyroid cancer cases and 1,200 deaths are reported in USA. The early phases of thyroid cancer are asymptomatic and, at the time of clinical presentation, the tumor has often escaped the confines of the thyroid gland, invading surrounding tissues or metastasizing to a distant location. In these patients, local control of the disease requires multiple surgical procedures that add significant morbidity and contribute to poor quality of life and unnecessary high mortality rates. Clinico-pathological parameters are insufficient to predict the behavior of thyroid cancer and, therefore, molecular predictors of prognosis are needed to provide an individualized approach to treatment.
In many tumors, oncogenesis is related to an acquired resistance to the normal growth inhibitory control mediated by Transforming Growth Factor ß (TGFß). We have previously shown that TGFß type II receptors (TβR-II) are markedly decreased, and that Smad proteins, responsible for the downstream transmission of TGFß signals, are abnormally expressed. These abnormalities lead to cyclin D1 overexpression, down-regulation of cyclin inhibitors, illicit entry into the G1 phase of the cell cycle, abnormal DNA repair, and oncogenesis. In fact, we have also reported that in thyroid cancer, levels of cyclin D1 correlate with clinical stage

Recently, RET translocations have been frequently observed in thyroid cancer but their true incidence, clinical significance, mechanisms of action, and relationship to other pathways are not known. Since RET translocations and abnormalities in proteins of the TGFβ cascade are common in thyroid cancer, these pathways are ideal candidates to establish "tumor molecular profiles" with relevance to prognosis. To validate the prospective use of this molecular information, results obtained in tissue from surgically removed tumors will be compared with results obtained from cells obtained from the same tumors prior to surgery. We will investigate TGFβ pathway abnormalities and RET oncogene translocations in surgically resected thyroid carcinomas at the RNA level. We will also investigate, at the protein level, the expression of members of the TGFβ pathway in tumor cells obtained prior to surgery and in tissue from the same tumors after surgery.

We expect to identify "molecular tumor profiles" that will provide information regarding the prognosis of each tumor. We will do the studies in two types of specimens: in cells obtained by small biopsies prior to surgery, and in the same tumors after they have been surgically removed. If the results are similar in these two types of material, the molecular prognostic information could be obtained from a very small amount of tissue, at the earliest time in the disease and with minimal discomfort to the patient.

BODY

A. To date, three cases have been collected for the purpose of this study. In all three cases, paraffin-embedded material and frozen sections have been prepared from tumor and adjacent non-neoplastic tissue. Furthermore, in two cases, cell blocks have been prepared from fine needle aspiration biopsy material. This material is being preserved for future testing once all testing conditions are optimized.

B. We have also begun the collection of archival paraffin-embedded material from thyroid cancer specimens operated at Moffitt Cancer Center. This material will be used to establish the optimal immunohistochemical conditions for TGFβ type II receptors and the family of Smad proteins. The appropriate antibodies and vendors have been identified. Once conditions are established, approximately 60 thyroid papillary carcinomas will be tested in a prospective manner.

C. Results from the immunohistochemistry studies will be correlated with PCR and RT-PCR analysis of the RET oncogene and its transcription products, and also with members of the TGFβ pathway. Nucleic acid material from tumoral and adjacent non-neoplastic cells will be independently collected from tumor frozen sections by Laser Capture Microdissection (LCM). Preliminary studies have been directed to identify optimal conditions for microdissection in paraffin-embedded tissues and frozen sections, and to test the quality of the nucleic acids obtained, by PCR and RT-PCR, using the actin gene as a target. These studies are currently under way.

D. The specific primers to detect the RET translocations and the Smad family of proteins have been designed and will be tested in the near future.

KEY RESEARCH ACCOMPLISHMENTS

The research project has just started. No accomplishments to report at this time

REPORTABLE OUTCOMES

No publications derived from this research at this time.

CONCLUSIONS

No research has been completed at this time.

REFERENCES

No references to report at this time

APPENDICES

Research in early stages. No appendix to add at this time.

APPENDIX K

Significance of Bax-Dependent Apoptosis in Prevention and Detection of Human Prostate and Lung Cancer

Q. Ping Dou

ANNUAL REPORT

Report Date: 10-05-2000

Proposal Title: Significance of Bax-Dependent Apoptosis in Prevention and Detection of Human Prostate and Lung Cancer

PI's Full Name: Q. Ping Dou

Phone: 813-632-1437
Fax: 813-979-6748
E-mail: douqp@moffitt.usf.edu

Proposal start date: 10/01/00 - 9/30/01

(1) INTRODUCTION:

The ubiquitin/proteasome-mediated protein degradation pathway plays an important role in regulating both cell proliferation and cell death (1-3). Programmed cell death (apoptosis) occurs in two physiological stages, commitment and execution, which are regulated by the Bcl-2 family proteins (4-6) and caspases (7-9), respectively. Decreased Bax expression or Bax/Bcl-2 ratio has been suggested as a predictive marker for metastasis and drug-resistance of human prostate and lung cancers (10-15), whereas increased Bax or Bax/Bcl-2 ratio was associated with prostate and lung cancer cell apoptosis in cultures and animal models (16-20). Therefore, discovery of the molecular mechanism regulating Bax expression has great clinical significance in prevention, detection and treatment of human prostate, lung and other cancers. We have generated the following *THREE HYPOTHESES*. (i) Bax degradation activity (BDA) levels determine Bax levels and therefore predict the progressive stages of prostate and lung cancer. (ii) BDA is also a diagnostic marker for response of prostate and lung cancer cells to proteasome inhibitor-induced apoptosis. (iii) A tumor-specific activity regulating BDA is a putative cancer susceptibility marker. There are *THREE SPECIFIC AIMS* proposed in this grant. Specific Aim 1 is to investigate whether BDA levels determine Bax levels in human prostate and lung cancer cell lines and tissue samples and whether BDA levels correlate cancer progressive stages. Specific Aim 2 is to investigate whether BDA levels predict response of human prostate and lung cancer cells to proteasome inhibitor-induced apoptosis in cultures. Specific Aim 3 is to look for the prostate and lung cancer risk marker that is involved in tumor-specific responsiveness to proteasome inhibitor-induced, Bax-/cytochrome c-dependent apoptosis.

(2) BODY:

Because this proposal is just funded most recently (10/01/2000), this section will be simplified.

Task 1. To investigate whether BDA levels determine Bax levels in human prostate and lung cancer cell lines and tissue samples and whether BDA levels correlate cancer progressive stages.

Most recently, we received the approval from DEPARTMENT OF THE ARMY for the use of human prostate and lung cancer tissue samples in the proposed studies. We are going to perform the proposed experiments very soon.

Currently, we have three human prostate cancer cell lines, PC-3, LNCaP and DU145, growing in the lab. It has been reported that Bax protein is expressed only in PC-3 and LNCaP, but not in DU145 cells (21-23), which provides an excellent model for studying the role of Bax in the process of proteasome inhibitor-mediated apoptosis induction. We found that treatment of LNCaP, but not DU145, cells with a proteasome inhibitor resulted in accumulation of Bax protein, suggesting that the proteasome inhibitor targets Bax degradation enzyme, the proteasome, *in vivo*. We will further investigate whether proteasome inhibition accumulates only Bax and other pro-apoptotic proteins, but not Bcl-2 and other anti-apoptotic proteins. We will focus on the comparison between Bax and Bcl-2 proteins.

We will repeat the proposed studies with several lines of human lung cancer cells.

Task 2. To investigate whether BDA levels predict response of human prostate and lung cancer cells to proteasome inhibitor-induced apoptosis in cultures

If the effects of a proteasome inhibitor on apoptosis are due to increased levels of Bax protein, then DU145 cells would be more resistant to proteasome inhibitor treatment than PC-3 and LNCaP cells. Indeed, our preliminary data demonstrated that DU145 cells were more resistant to the proteasome inhibitor LLL-induced apoptosis than both LNCaP and PC-3 cells. After LLL treatment, Δ50% of PARP was cleaved in PC-3 or LNCaP cells between 12-24 h, whereas only <10% PARP cleaved in DU145 cells at 24 h (Fig. 1).

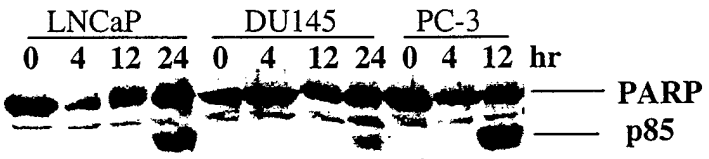


Fig. 1. LNCaP, DU145 and PC-3 cells (0 h) were treated with 50 μM of LLL, followed by measurement of apoptosis-specific PARP cleavage.

Task 3. To look for the prostate and lung cancer risk marker which is involved in tumor-specific response to proteasome inhibitor-induced, Bax-/cytochrome c-dependent apoptosis.

Human normal and cancer prostate and lung tissues will be obtained very soon for the proposed studies.

Resistance of normal WI-38 cells to release cytochrome c upon proteasome inhibitor treatment. Previously we reported that tumor and SV40-transformed cells were much more sensitive to proteasome inhibitor-induced apoptosis than normal WI-38 cells (24) and that tumor cell death induced by proteasome inhibitors was associated with cytochrome c release and dependent on caspase activation (25). We hypothesized that resistance of normal human cells to proteasome inhibitor-induced apoptosis is due to failure or resistance of these cells to release mitochondrial cytochrome c into the cytosol. To test this hypothesis, human normal WI-38 and SV-40-transformed WI-38 (VA-13) cells were treated with the tripeptidyl proteasome inhibitor LLnL for up to 24 h, followed by measurement of apoptosis-specific PARP cleavage and cytosolic cytocrome c levels.

About 30% of PARP protein was cleaved in VA-13 cells treated with LLnL for 8 h, and almost all the PARP protein was cleaved by 24 h (Fig. 2A, lanes 1-4). In contrast, when WI-38 cells were treated under the identical conditions, no PARP cleavage was detected at 8 h, and even after 24 h, only very low levels of the PARP cleavage fragment (PARP/p85) were detected (Fig. 2A, lanes 5-8). To measure release of mitochondrial cytochrome c into the cytosol in both cell lines, cytosolic fractions were prepared and used for Western blot assay with a specific cytochrome c antibody. Levels of cytosolic cytochrome c were significantly increased in LLnL-treated VA-13 cells, which started at 8 h (Fig. 2B, lanes 1-6). Levels of cytosolic cytochrome c were low in untreated WI-38 cells (Fig. 2B, lane 7), remained low after LLnL treatment for up to 16 h, and slightly increased only after 24 h-treatment (Fig. 2B, lanes 8-12). The observed different kinetics of cytochrome c release in both VA-13 and WI-38 cell lines were not due to unevenly loaded samples since the same cytochrome c antibody also detected a ~40 kDa protein band with unknown nature in these cytosolic fractions whose levels were relatively unchanged (Fig. 2B). Importantly, the kinetics of cytochrome c release in both VA-13 and WI-38 cells matched well that of PARP cleavage (Fig. 2B vs. 2A). Therefore, a proteasome inhibitor-inducible pathway that controls cytochrome c release is suppressed in normal WI-38 cells.

Resistance of normal WI-38 cells to accumulate mitochondrial Bax protein after proteasome inhibitor treatment. Our previous studies suggested that before or associated with cytochrome c release in Jurkat T cells treated with a proteasome inhibitor, Bax was accumulated to mitochondria (25). We then tested the idea whether resistance of WI-38 cells to release of cytochrome c by proteasome inhibition (Fig. 2B) was associated with failure of these cells to accumulate Bax protein in mitochondria. We first measured Bax protein levels using whole cell extracts prepared from VA-13 and WI-38 lines. LLnL treatment of VA-13 cells increased levels of Bax and a Bax-related, 55 kDa polypeptide (Fig. 3A). By 24 h, levels of both Bax and p55 were increased by 3- to 4-fold (Fig. 3A). A similar p55 was found in both Jurkat T cells treated with a proteasome inhibitor and high-grade prostate cancer tumor samples, which can be detected by antibodies to both Bax and ubiquitin proteins (25). These data suggest that p55 may contain ubiquitinated Bax. In contrast to VA-13 cells, Bax protein levels in WI-38 cells were not increased up to 16 h-treatment with LLnL, and only slightly increased (< 2-fold) after 24 h-treatment (Fig. 3B). A similar p55 was also detected in WI-38 cells, whose levels were slightly increased by LLnL treatment for 24 h, but not shorter (Fig. 3B). An extra band of ~35 kDa with unknown nature was found only in WI-38, but not in VA-13, cells (Fig. 3B vs. 3A). The basal levels of Bcl-2 protein were lower in WI-38 than VA-13 cells (Fig. 3C, lanes 5 vs. 1). Bcl-2

protein levels were slightly increased in VA-13 cells, while decreased in WI-38 cells, after a 24 h-treatment with LLnL (Fig. 3C).

We then measured Bax protein levels in both cytosolic and mitochondrial fractions of VA-13 and WI-38 cells. Similar to Jurkat T cells (25), when the transformed VA-13 cells were treated with LLnL, cytosolic Bax levels were decreased, associated with increased levels of mitochondrial Bax protein (Fig. 4, lanes 4-6 vs. lanes 10-12). However, when WI-38 cells were analyzed under identical conditions, little or no mitochondrial Bax protein was detected although cytosolic Bax protein levels were decreased (Fig. 4, lanes 7-9 vs. 1-3). Therefore, WI-38 cells failed to accumulate Bax protein to mitochondria after proteasome inhibitor treatment, which was associated with resistance of these cells to release mitochondrial cytochrome c and induce apoptosis (Fig. 2).

We are going to measure whether normal WI-38 cells fail to accumulate ubiquitinated forms of Bax protein upon proteasome inhibition. We will also measure levels of Bax-associated Bcl-2 protein in both transformed and normal human fibroblasts after proteasome inhibitor treatment. Finally, we will determine levels of proteasome-mediated Bax degradation activity in both cell lines.

(3) KEY RESEARCH ACCOMPLISHMENTS:

a. Bax is required for proteasome inhibitor-induced apoptosis in human prostate cancer cells.

b. Resistance of human normal lung cells to proteasome inhibitor treatment is associated with failure of these cells to accumulate Bax to mitochondria.

(4) REPORT OUTCOMES:

We are performing additional experiments for two manuscripts.

(5) CONCLUSIONS:

a. When prostate cancer cells containing wild-type Bax protein (such as LNCaP and PC-3) are treated with a proteasome inhibitor, Bax protein is accumulated, followed by induction of apoptosis. Prostate cancer cells without Bax protein (such as DU145) are more resistant to proteasome inhibitor-induced apoptosis than prostate cancer cells containing wild-type Bax protein.

b. Resistance of normal human lung WI-38 cells to accumulate mitochondrial Bax protein after proteasome inhibitor treatment is associated with failure of these cells to release cytochrome c and undergo apoptosis.

(6) REFERENCES:

1. Hochstrasser, M. Ubiquitin, proteasomes, and regulation of intracellular protein degradation. Curr. Opin. Cell Biol. 1995; 7: 215-223.
2. Ciechanover, A. The ubiquitin-proteasome proteolytic pathway. Cell 1994; 79: 13-21.
3. Dou QP, Li B. Proteasome inhibitors as potential novel anticancer agents. Drug Resist Updates 1999; 2: 215-223.
4. Reed J.C. Bcl-2 and the Regulation of Programmed Cell Death. J. Cell Biol. 1994; 124: 1-6.
5. Green, D. R. and Reed, J. C. Mitochondria and Apoptosis. Science 1998; 281: 1309-1312.
6. Cory, S. and Adams, J. M. The Bcl-2 Protein Family: Arbiters of Cell Survival. Science 1998; 281: 1322-1326.
7. Earnshaw, W. C. Nuclear Changes in Apoptosis. Curr. Opin. Cell Biol. 1995; 7: 337-343
8. Steller, H. Mechanisms and genes of cellular suicide. Science 1995; 267: 1445-1449.
9. Thornberry, N. A. and Lazebnik, Y. Caspases: Enemies Within. Science 1998, 281: 1312-1316
10. van Brussel JP, Mickisch GH. Circumvention of multidrug resistance in genitourinary tumors. Int J Urol. 1998; 5: 1-15.
11. Mackey TJ, Borkowski A, Amin P, Jacobs SC, Kyprianou N. bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with prostate cancer. Urology 1998; 52: 1085-1090.
12. McConkey DJ, Greene G, Pettaway CA. Apoptosis resistance increases with metastatic potential in cells of the human LNCaP prostate carcinoma line. Cancer Res. 1996; 56: 5594-5599.
13. Nishio K, Nakamura T, Koh Y, Suzuki T, Fukumoto H, Saijo N. Drug resistance in lung cancer. Curr Opin Oncol 1999; 11: 109-115.
14. Brambilla E, Negoescu A, Gazzeri S, Lantuejoul S, Moro D, Brambilla C, Coll JL. Apoptosis-related factors p53, Bcl-2, and Bax in neuroendocrine lung tumors. Am J Pathol. 1996; 149: 1941-1952.
15. Brambilla E, Gazzeri S, Lantuejoul S, Coll JL, Moro D, Negoescu A, Brambilla C. p53 mutant immunophenotype and deregulation of p53 transcription pathway (Bcl-2, Bax, and Waf1) in precursor bronchial lesions of lung cancer. Clinical Cancer Res 1998; 4: 1609-1618.
16. Garde SV, Basrur VS, Li L, Finkelman MA, Krishan A, Wellham L, Ben-Josef E, Haddad M, Taylor JD, Porter AT, Tang DG. Prostate secretory protein (PSP94) suppresses the growth of androgen-independent prostate cancer cell line (PC3) and xenografts by inducing apoptosis. Prostate 1999; 38: 118-125.
17. Perlman H, Zhang X, Chen MW, Walsh K, Buttyan R. An elevated bax/bcl-2 ratio correlates with the onset of prostate epithelial cell apoptosis. Cell Death Diff 1999; 6: 48-54.
18. Guo Y, Kyprianou N. Restoration of transforming growth factor beta signaling pathway in human prostate cancer cells suppresses tumorigenicity via induction of caspase-1-mediated apoptosis. Cancer Res 1999; 59: 1366-1371.
19. Lian F, Li Y, Bhuiyan M, Sarkar FH. p53-independent apoptosis induced by genestein in lung cancer cells. Nutr Cancer 1999; 33: 125-131.
20. Coll JL, Negoescu A, Louis N, Sachs L, Tenaud C, Girardot V, Demeinex B, Brambilla E, Brambilla C, Favrot M. Antitumor activity of bax and p53 naked gene transfer in lung cancer: in vitro and in vivo analysis. Human Gene Ther 1998; 9: 2063-2074.
21. Haldar S, Chintapalli J, Croce CM Taxol induces bcl-2 phosphorylation and death of prostate cancer cells. *Cancer Res* 1996;56:1253-5.

22. Rokhlin OW, Bishop GA, Hostager BS, Waldschmidt TJ, Sidorenko SP, Pavloff N, Kiefer MC, Umansky SR, Glover RA, Cohen MB. Fas-mediated apoptosis in human prostatic carcinoma cell lines. *Cancer Res* 1997;57:1758-68.
23. Tang DG, Li L, Chopra DP, Porter AT. Extended survivability of prostate cancer cells in the absence of trophic factors: increased proliferation, evasion of apoptosis, and the role of apoptosis proteins. *Cancer Res* 1998;58:3466-79.
24. An, B., Goldfarb, R. H., Siman, R., Dou, Q. P. Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. Cell Death & Diff. 1998; 5: 1062-1075.
25. Li B, Dou QP. 2000. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. Proc. Natl. Acad. Sci. USA 2000; 97: 3850-3855.

(7) APPENDICES:

Three figures and one copy of CV are enclosed.

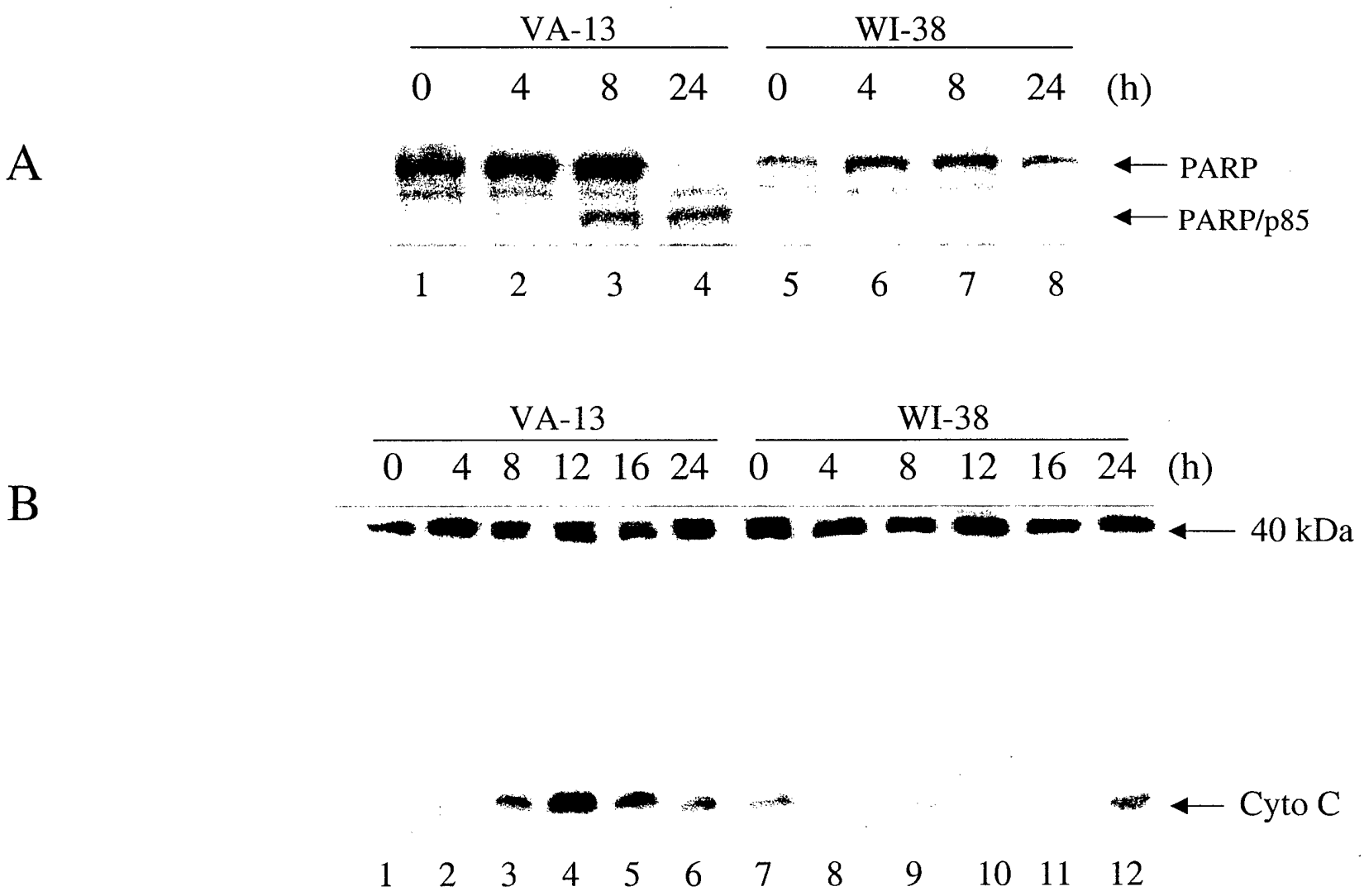


Fig. 2. Both WI-38 and VA-13 cell lines were treated with proteasome inhibitor LLnL (50 uM) for up to 24 h, followed by measurement of PARP cleavage (A) and cytochrome c release (B). (PI: Q. Ping Dou)

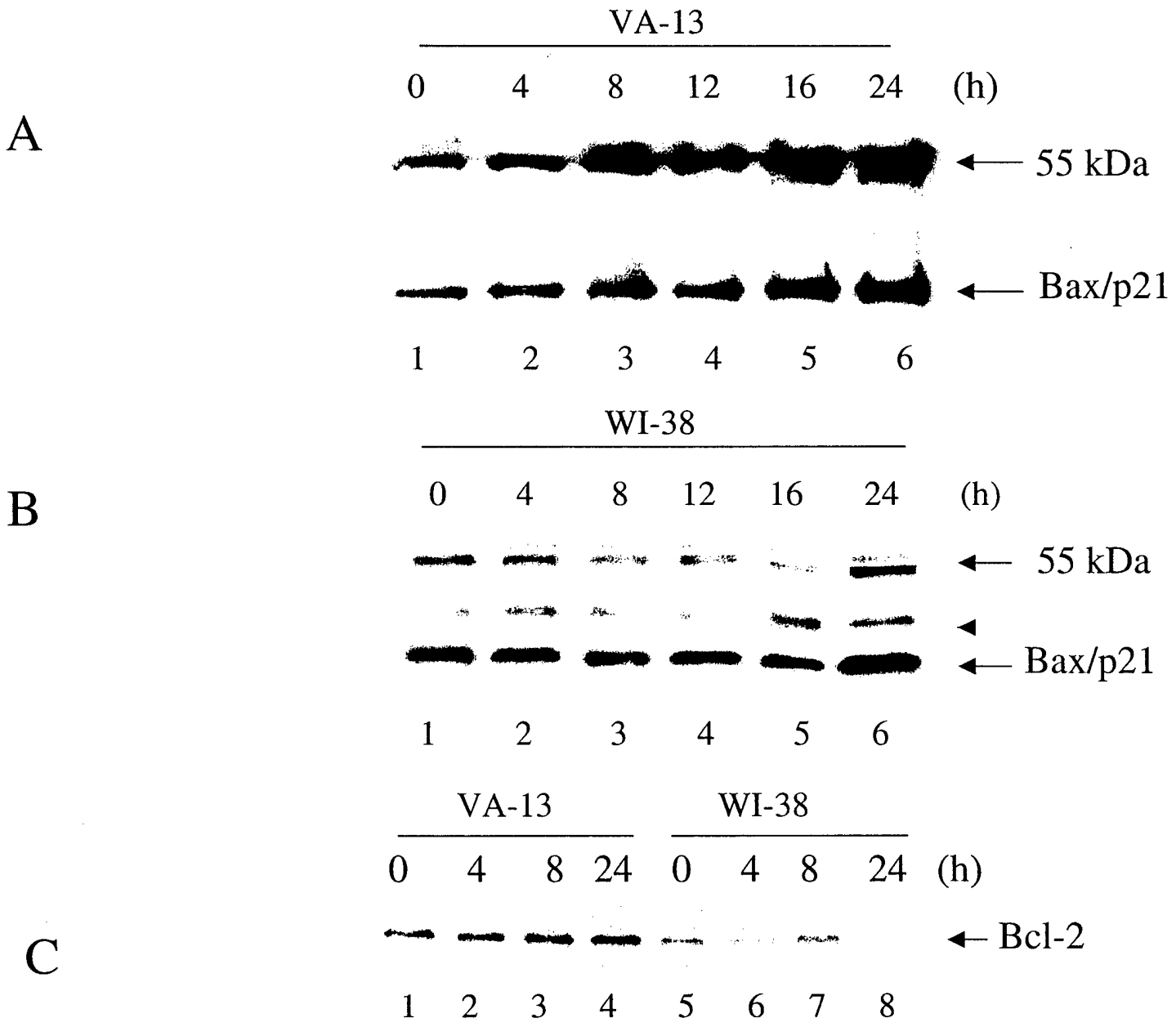


Fig. 3. Levels of Bax (A, B) and Bcl-2 (C) proteins were measured in the experiment described in figure 2.

(PI; Q. Ping Dou)

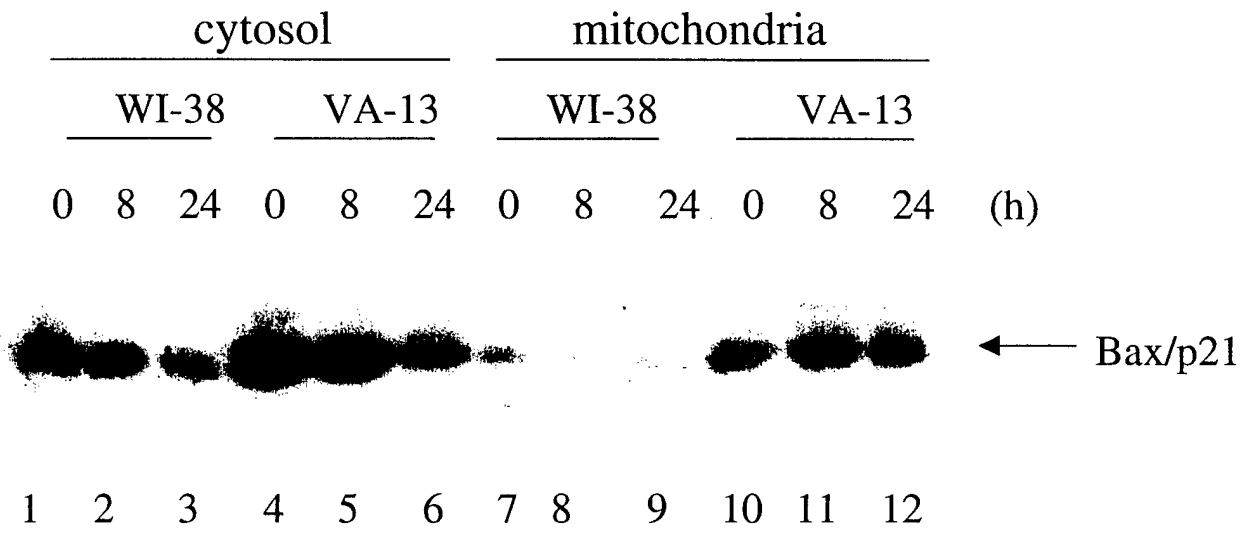


Fig. 4. Both WI-38 and VA-13 cell lines were treated with proteasome inhibitor LLnL (50 uM) for up to 24 h, followed by isolation of mitochondrial and cytosolic fractions. The isolated fractions were used for Western Blot assay using a specific Bax antibody.

PI: Q. Ping Dou

9-30-2000

CURRICULUM VITAE

Q. PING DOU

PII Redacted

I. PERSONAL INFORMATION

[redacted]

Marital Status: Married (Wife: Anna Wang; Children: Eva and Dan Dou)

Business Address: Drug Discovery Program, MRC 1259C
H. Lee Moffitt Cancer Center & Research Institute, and
Department of Biochemistry and Molecular Biology
College of Medicine
University of South Florida
12902 Magnolia Drive
Tampa, FL 33612-9497
Telephone: 813-632-1437
Fax: 813-979-6748
e-mail: douqp@moffitt.usf.edu

[redacted]

II. EDUCATION

1988-1992 Postdoctoral Research Fellow, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and Division of Cell Growth and Regulation, Dana-Farber Cancer Institute, Boston, MA (Mentor: Arthur B. Pardee). Researched in the areas of cell cycle regulation, transcription, proliferation and cancer

1988 Ph.D. in Chemistry, Rutgers University, Piscataway, NJ (Mentor: Kuang Yu Chen).

1981 B.S. in Chemistry, Shandong University, Jinan, Shandong, People's Republic of China

III. EMPLOYMENT

2000-present Associate Professor, Interdisciplinary Oncology Program (IOP), University of South Florida College of Medicine, Tampa, Florida.

1998-present Associate Professor, Department of Biochemistry and Molecular Biology, University of South Florida College of Medicine, Tampa, Florida. Researched in signal transduction pathways controlling cell growth and death, discovery of new chemotherapeutic targets, and development of selective, effective anticancer drugs

1998-present Member in Residence, Drug Discovery Program, H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida

1998-present Member, the Institute for Biomolecular Science, University of South Florida, Tampa, Florida

1993-1998 Assistant Professor, Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA. Researched in the areas of molecular mechanisms for anticancer drug-induced programmed cell death (apoptosis) and drug resistance

1997-1998 Assistant Professor, Biochemistry and Molecular Genetics Graduate Training Program, Interdisciplinary Biomedical Graduate Program, University of Pittsburgh School of Medicine, Pittsburgh, PA

1993-1998 Member, Experimental Therapeutic Program, University of Pittsburgh Cancer Institute, Pittsburgh, PA

1992-1993 Instructor, Department of Medicine, Harvard Medical School, at Dana-Farber Cancer Institute and Beth Israel Hospital. Researched in mechanisms of tumor growth and cellular aging

1986-1988 Research Assistant, Department of Chemistry, Rutgers University. Researched in the areas of cell cycle regulation, post-translational modifications and polyamines

1983-1986 Teaching Assistant, Department of Chemistry, Rutgers University

1987-1988 Drug Specialist, Psychiatric Diagnostic Laboratories of America Inc., Piscataway, NJ. Researched in monoamine oxidase in human blood and platelet samples, and in analysis of drugs by using HPLC, GC and other equipment

IV. RESEARCH PROGRAM

One of my laboratory's research interests is the signal transduction pathway regulating apoptosis and drug resistance and involvement of cell cycle regulators in these processes. We have found that during the process of apoptosis, the tumor suppressor retinoblastoma protein (RB) becomes dephosphorylated and immediately cleaved, mediated by an activated protein-serine/threonine phosphatase and caspase (interleukin 1β-converting enzyme-like protease), respectively. Failure to induce these RB changes is tightly associated with drug resistance in human cancer cells. We have mapped the caspase cleavage site located in RB and made a point-mutation. We are going to test the functional significance of RB cleavage in apoptosis by using these mutations-containing cells. Another investigation in my laboratory is the role of proteasome in apoptosis. We have found that proteasome inhibitors are able to rapidly induce apoptosis in *all* the human cancer cell lines tested, including leukemia, breast, prostate, bone, brain and head and neck, *but*

not in normal human fibroblasts. Possible molecular mechanisms involve, at least, accumulation of the cyclin-dependent kinase inhibitor p27 and the apoptosis inducer Bax. Presently, we are (i) evaluating the ability of novel proteasome inhibitors to inhibit human tumor growth in animal models, (ii) investigating levels of the proteasome activity in human normal and tumor breast tissues, and (iii) studying the signal transduction pathway responsible for why cancer cells are selectively killed by inhibition of the proteasome and why normal cells are resistant to proteasome inhibitor treatment. All molecular biology and molecular pharmacology studies are directed toward the discovery of new chemotherapeutic targets.

Key words: Drug discovery, cell cycle, apoptosis, oncogenes, tumor suppressor genes, cyclin-dependent kinases, cdk inhibitors, phosphatases, proteasome, drug resistance

V. HONORS AND AWARDS

2000	Moffitt's Cancer Center Director's Award (for the article published by Li B and Dou QP in Proc. Natl. Acad. Sci. USA, 2000; 97: 3850-3855). Moffitt Cancer Center & Research Institute.
2000	Acceptance to National Cancer Institute Supported LINK Program (Leader In New Knowledge) at Moffitt Cancer Center & Research Institute (for Marie E. Bosley; mentor: Q. Ping Dou). Moffitt Cancer Center & Research Institute.
2000	Department of the Army Advanced Cancer Detection Center Research Grant. Moffitt Cancer Center & Research Institute.
1999	Cheryl L. Fattman, Ph.D. Graduation with Honor from University of Pittsburgh (mentor: Q. Ping Dou)
1999-present	Ad Hoc Reviewer, Merit Review Subcommittee for Oncology, the Veterans Affairs Medical Research Programs, US Department of Veterans Affairs
1997	Award for the Best Abstract. 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997
1997	Chairman for Session of Clinical Oncology/Apoptosis. 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997
1997-present	Invited member of the Editorial Board of *Frontiers In Bioscience*
1997	The Best Poster Presentation (An B *et al.*), Scientific Retreat, Department of Pharmacology, University of Pittsburgh School of Medicine
1997-1999	a Predoctoral Trainingship in Breast Cancer Biology and Therapy from the United States Army Medical Research, Development, Acquisitions, and Logistics Command (to Cheryl L. Fattman)
1996-present	Invited member of the Editorial Board of the *Oncology Reports*
1996-2001	NIH FIRST Award, 1 R29 AG 13300-01A1
1995-1997	NIH Director James A. Shannon Award, 1 R55 AG/OD13300-01
1996-1997	Breast Cancer Pilot Grant. University of Pittsburgh Cancer Institute
1995	Co-Discussion Leader, University of Pittsburgh Cancer Institute Scientific Retreat
1993-1994	American Cancer Society Institutional Research Grant. University of Pittsburgh Cancer Institute
1992	Barr Program Small Grant. Dana-Farber Cancer Institute
1991	Biochemical Research Support Grant. Dana-Farber Cancer Institute

1988 Summer Research Prize in recognition of outstanding accomplishments in research. Rutgers University

VI. PROFESSIONAL SOCIETY MEMBERSHIPS

American Association for Cancer Research, Inc.
American Association for the Advancement of Science
American Society for Biochemistry and Molecular Biology
American Society for Pharmacology and Experimental Therapeutics
Society of Chinese Bioscientists in America
New York Academy of Sciences (1995-1998)

VII. PUBLICATIONS

A. Refereed Journals

1. Chen KY and Dou QP. NAD^+ stimulated the spermidine-dependent hypusine formation on the 18,000-dalton protein in cytosolic lysates derived from NB-15 mouse neuroblastoma cells. FEBS Letters, 1988; 229: 325-328
2. Chen KY and Dou QP. Isolation and characterization of an 18,000-dalton hypusine-containing protein from NB-15 mouse neuroblastoma cells. Biochem. Biophys. Acts, 1988; 971: 21-28
3. Dou QP and Chen KY. Two hypusine-containing proteins identified by metabolic labeling in chick embryo fibroblasts. J. Chin. Chem. Soc., 1989; 36: 443-450
4. Dou QP and Chen KY. Characterization and reconstitution of a cell-free system for NAD^+-dependent deoxyhypusine formation on the 18 kDa elF-4D precursor. Biochem. Biophys. Acts, 1990; 1036: 128-137
5. Bradley DW, Dou QP, Fridovich-Keil JL and Pardee AB. Transformed and non-transformed cells differ in stability and cell cycle regulation of a binding activity to the thymidine kinase promoter. Proc. Natl. Acad. Sci. USA, 1990; 87: 9310-9314
6. Dou QP, Fridovich-Keil JL and Pardee AB. Inducible proteins binding to the murine thymidine kinase promoter in late G1/S phase. Proc. Natl. Acad. Sci. USA, 1991; 88: 1157-1161
7. Fridovich-Keil JL, Gudas JM, Dou QP, Bouvard I and Pardee AB. Growth-responsive expression from the murine thymidine kinase promoter: Genetic analysis of DNA sequences. Cell Growth. Diff., 1991; 2: 67-76
8. Dou QP, Markell PJ, and Pardee AB. Thymidine kinase transcription is regulated at G1/S phase by a complex that contains retinoblastoma-like protein and cdc2 kinase. Proc. Natl. Acad. Sci. USA, 1992; 89: 3256-3260
9. Dou QP, Levin AH, Zhao S, and Pardee AB. Cyclin E and cyclin A as candidates for the restriction point protein. Cancer Res., 1993; 53: 1493-1497
10. Dou QP, Zhao S, Levin AH, Wang J, Helin K, and Pardee AB. G1/S-regulated E2F-containing complexes bind to the mouse thymidine kinase gene promoter. J. Biol. Chem., 1994; 269: 1306-1313

11. Dou QP, Molnar G, and Pardee AB. Cyclin D1/cdk2 kinase is present in a G1 phase-specific protein complex Yi1 that binds to the mouse thymidine kinase gene promoter. Biochem. Biophys. Res. Commun., 1994; 205: 1859-1868
12. Dou QP, An B and Will PL. Induction of a retinoblastoma phosphatase activity by anticancer drugs accompanies p53-independent G1 arrest and apoptosis. Proc. Natl. Acad. Sci. USA, 1995; 92: 9019-9023
13. Dou QP, An B and Yu C. Activation of cyclin E-dependent kinase by DNA-damaging signals during apoptosis. Biochem. Biophys. Res. Commun., 1995; 214: 771-780
14. Dou QP and Lui VWY. Failure to dephosphorylate retinoblastoma protein in drug resistant cells. Cancer Res., 1995; 55: 5222-5225
15. An B and Dou QP. Cleavage of retinoblastoma protein during apoptosis: an interleukin 1β-converting enzyme-like protease as candidate. Cancer Res., 1996; 56: 438-442
16. Dou QP, Pardee AB and Keyomarsi K. Cyclin E—a better prognostic marker for breast cancer than cyclin D? Nature Medicine, 1996; 2: 254
17. An B, Jin J-R, Lin P and Dou QP. Failure to activate interleukin 1β-converting enzyme-like proteases and to cleave retinoblastoma protein in drug-resistant cells. FEBS Letters; 1996; 399: 158-162
18. Dou QP, An B, Antoku K and Johnson DE. Fas stimulation induces RB dephosphorylation and proteolysis that is blocked by inhibitors of the ICE protease family. J. Cell. Biochem., 1997; 64: 586-594
19. Molnar GM, Crozat A, Kraeft S-K, Dou QP, Chen LB and Pardee AB. Association of the mammalian helicase MAH with the pre-mRNA splicing complex. Proc. Natl. Acad. Sci. USA, 1997; 94: 7831-7836
20. Fattman CL, An B and Dou QP. Characterization of interior cleavage of retinoblastoma protein in apoptosis. J. Cell. Biochem., 1997; 67: 399-408
21. An B, Dineley KE, Zhang LL, Termin TA, Meijer L and Dou QP. Involvement of RB kinases and phosphatases in life and death decisions. Oncology Reports, 1997; 4: 1129-1134
22. An B, Johnson DE, Jin J-R, Antoku K and Dou QP. Bcl-2- and CrmA-inhibitable dephosphorylation and cleavage of retinoblastoma protein during etoposide-induced apoptosis. Intl. J. Mol. Med., 1998; 1: 131-136
23. Fattman CL, An B, Sussman L and Dou QP. p53-independent dephosphorylation and cleavage of retinoblastoma protein during tamoxifen-induced apoptosis in human breast carcinoma cells. Cancer Lett., 1998; 130: 103-113
24. An B, Goldfarb RH, Siman R and Dou QP. Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. Cell Death & Diff., 1998; 5: 1062-1075
25. Dou QP, McGuire TF, Peng Y and An B. Proteasome inhibition leads to significant reduction of Bcr-Abl expression and subsequent induction of apoptosis in K562 human chronic myelogenous leukemia cells. J. Pharm. Exp. Ther., 1999; 289: 781-790
26. Reichert TE, Nagashima S, Kashii Y, Stanson J, Gao G, Dou QP and Whiteside TL. Interleukin-2 expression in human carcinoma cell lines and its role in cell cycle progression. Oncogene, 2000; 19: 514-525
27. Li B and Dou QP. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. Proc. Natl. Acad. Sci. USA, 2000; 97: 3850-3855

28. Gao G and Dou QP. G1 phase-dependent Bcl-2 expression correlates with chemoresistance of human cancer cells. Mol. Pharm., in press
29. Sun J, Li B, Lee C-S, Nam S, Coppola D, Hamilton AD, Dou QP and Sebti SM. CEP1612, a dipeptidyl proteasome inhibitor, induces $p21^{WAF1}$ and $p27^{KIP1}$ expression and apoptosis and inhibits the growth of the human lung adenocarcinoma A-549 in nude mice. Cancer Res., in revision
30. Smith DM and Dou QP. Induction of S phase-associated leukemia cell apoptosis by green tea polyphenol (-)-Epigallocatechin. Submitted to Leukemia Res.
31. Fattman CL, Delach S, Dou QP and Johnson DE. Sequential two-step cleavage of the retinoblastoma protein by caspase-3 during etoposide-induced apoptosis. Submitted to Cancer Res.
32. Gao G and Dou QP. N-Terminal Cleavage of Bax Protein by a Mitochondrial Calpain Activity Generates a Potent Proapoptotic 18 KD Fragment That Induces Bcl-2-Independent Cytochrome C Release and Apoptotic Cell Death. J Cell. Biochem., in press
33. Nam S, Smith DM and Dou QP. Inhibition of proteasome activity in vitro and in vivo by ester bond-containing tea polyphenols. JBC, in revision

B. Invited Reviews and Book Chapters

1. Boothman DA, Lee S, Trask DK, Dou QP and Hughes EN. X-ray-inducible proteins and genes in human cells. In: UCLA Symposia on Molecular and Cellular Biology: Ionizing Radiation Damage to DNA: Molecular Aspects, Wiley-Liss, Inc., Publication 1990; pp. 309-317
2. Fridovich-Keil JL, Gudas JM and Dou QP. Regulation of gene expression in late G1: What can we learn from thymidine kinase? In: Perspectives on Cellular Regulation: From Bacteria To Cancer, Wiley-Liss, Inc., Publication 1991; pp. 265-277
3. Dou QP and Pardee AB. Transcriptional activation of thymidine kinase, a marker for cell cycle control. Progress in Nuclear Acid Research and Molecular Biology, 1996; 53: 197-217
4. Pardee AB, Keyomarsi K and Dou QP. Regulation of the cell cycle by kinases and cyclins. In: Colony-Stimulating Factors, Molecular and Cellular Biology, second edition, revised and expended (edited by J.M. Garland, P.J. Quesenberry and D.J. Hilton), Marcel Dekker. 1997; pp. 71-95
5. Dou QP. Putative roles of retinoblastoma protein in apoptosis. Apoptosis, 1997; 2: 5-18
6. Dou QP and An B. RB and apoptotic cell death. Frontiers In Bioscience, 1998; 3: d419-430
7. Dou QP and Li B. Proteasome inhibitors as potential novel anticancer agents (invited review). Drug Resistance Updates, 1999; 2: 215-223
8. Dou QP and Nam S. Proteasome inhibitors and Their Therapeutic Potential (invited review). Expert Opinion on Therapeutic Patents, 2000; 10: 1263-1272
9. Smith DM, Gao G and Dou QP. Regulation of tumor cell apoptotic sensitivity during the cell cycle (invited review). Intl. J. Mol. Med., 2000, in press
10. Whiteside TL, Reichert TE and Dou QP. Interleukin-2 and its receptors in human solid tumors: immunology and clinical significance. Submitted

VIII. ABSTRACTS

1. Dou QP and Chen KY. Isolation of an 18,000-dalton hypusine-containing protein from cultured mouse neuroblastoma cells. Fed. Proc., 1987; 46:961a
2. Chen KY and Dou QP. Polyamines metabolically labeled two cellular proteins in fibroblasts isolated from chick embryos. Fed. Proc., 1987; 46:962a
3. Dou QP and Chen KY. NAD^+ significantly stimulated hypusine formation on the 18,000-dalton protein (elF-4D) by polyamines in a cytosolic lysate isolated from NB-15 mouse neuroblastoma cells. FASEB Journal 2, 1988; 7360
4. Dou QP and Chen KY. Isolation and reconstitution of the hypusine formation of elF-4D *in vitro* in mouse neuroblastoma cells. J. Cell. Biol., 1989; 107:3633
5. Dou QP, Markell PJ and Pardee AB. Cdc2 kinase is copurified with a DNA-binding protein of 60 kDa that is involved in a G1/S-specific DNA-protein complex. Cold Spring Harbor Laboratory, 56th Symposium on Quantitative Biology, The Cell Cycle, 1991; 56
6. Dou QP, Markell PJ and Pardee AB. Thymidine kinase transcription is regulated at G1/S by a complex that contains retinoblastoma-like protein and cdc2 kinase. Aging Gordon Conference, 1992
7. Dou QP. Molecular Biology of Altered G1/S Regulation in Cancer. Pittsburgh Breast Cancer Conference, 1993
8. Dou QP. Association of cyclin D-dependent kinases with a G1/S phase-regulated DNA-binding protein complex Yi which is different from E2F. 85th Annual Meeting of the American Association for Cancer Research, San Francisco, California, 1994
9. An B, Yu K, Will P, and Dou QP. Induction of cyclin E-dependent kinase during Ara-C-induced apoptosis. Pittsburgh Cancer Institute scientific Retreat, Johnstown, PA, 1994
10. Dou QP. Induction of an RB phosphatase activity by anticancer drugs accompanies p53-independent G1 arrest and apoptosis. Gordon Research Conferences: Cell Death, New London, NH, 1995
11. An B., Will PL and Dou QP. Induction of an RB phosphatase activity by anticancer drugs accompanies p53-independent G1 arrest and apoptosis. UPCI Scientific Retreat, Johnstown, PA, 1995
12. Lui V and Dou QP. Failure to induce RB dephosphorylation is tightly associated with drug resistance. UPCI Scientific Retreat, Johnstown, PA, 1995
13. An B, Jin JR and Dou QP. Cleavage of retinoblastoma protein (RB) by a member of interleukin 1β-converting enzyme (ICE) family at the initiation of apoptotic execution. AACR 87th annual meeting, Washington, D.C., 1996; 96
14. An B and Dou QP. Failure to activate ICE-like proteases and cleave retinoblastoma protein in drug-resistant cells. UPCI 8th Annual Scientific Retreat, Johnstown, PA, 1996
15. Dineley K and Dou QP. Induction of apoptosis-associated RB dephosphorylation and cleavage by protein kinase C inhibitors. UPCI 8th Annual Scientific Retreat, Johnstown, PA, 1996
16. Fattman CL and Dou QP. Apoptosis-associated RB and PARP cleavage involves distinct ICE-like proteases. UPCI 8th Annual Scientific Retreat, Johnstown, PA, 1996
17. Fattman CL and Dou QP. Distinct ICE-like proteases mediate cleavage of retinoblastoma protein and poly(ADP-ribose) polymerase during apoptosis. AACR 88th annual Meeting, San Diego, California, April 12-16, 1997.
18. Dou QP, An B and Fattman CL. Retinoblastoma protein and the regulation of apoptosis. 7th SCBA International Symposium, Toronto, Canada, July 6-11, 1997.

19. Dou QP. Fattman CL, An B. Putative roles of retinoblastoma protein in apoptosis. 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997
20. Fattman CL, An B, Zhang LL, Dineley K and Dou QP. Dephosphorylation and cleavage of the retinoblastoma protein during p53-dependent and -independent apoptosis of human breast carcinoma cells. Department of Defense, U.S. Medical Research and Material Command, Breast Cancer Research Program: An Era of Hope, Washington, D.C., October 31-November 4, 1997.
21. Dou QP. Cell cycle checkpoint proteins as apoptosis therapeutic targets. NATO Advanced Research Workshop at H. Lee Moffitt Cancer Center and Research Institute University of South Florida, Protein-Protein and Protein-Lipid Interactions in Signal Transduction: Use of Small Synthetic Molecules as probes and Therapeutic Agents, Clearwater Beach, Florida, December 5-9, 1997
22. Johnson DE, Rabinovitz A, Dou QP, Delach SM and Fattman CL. VP-16 treatment of T-leukemic cells results in activation of Cathepsins D and L via a caspase-dependent pathway. 1999 Annual Meeting of the American Society of Hematology, New Orland, Louisiana, December 2-7, 1999
23. Dou QP and Li B. Bax degradation by the ubiquitin/proteasome-dependent pathway: involvement in tumor survival and progression. AACR 91st Annual Meeting, San Francisco, CA, April 1-5, 2000
24. Dou QP, Gao G and Zhang X. Insulin/IGF-I receptor-mediated signal transduction pathway regulates G_1 phase-dependent Bcl-2 expression and tumor chemoresistance. AACR 91st Annual Meeting, San Francisco, CA, April 1-5, 2000
25. Sun J, Li B, Lee C-S, Nam S, Coppola D, Hamilton AD, Dou QP and Sebti SM. The dipeptidyl proteasome inhibitor LCS-640 inhibits growth and induces apoptosis of the human lung adenocarcinoma A-549 xenografts in nude mice. AACR 91st Annual Meeting, San Francisco, CA, April 1-5, 2000
26. Gao G and Dou QP. G1 phase-dependent Bcl-2 expression correlates with chemoresistance of human cancer cells. 29th Annual Scientific Meeting of the International Society for Experimental Hematology, Tampa, FL, July 8-11, 2000
27. Smith DM and Dou QP. Green tea targets human tumor cell DNA synthesis and consequently induces apoptosis. Poster presentation. New Molecular Targets for Cancer Therapy, St. Petersburg Beach, FL, October 14-17, 2000

IX. SYMPOSIUM AND INVITED SEMINARS

1. Dou QP. Thymidine kinase as a marker of S-phase regulation. Cancer Chemotherapy Gordon Conference, 1991
2. Dou QP, and Pardee AB. Proliferation control at the G1/S boundary. 18th Meeting of the European Study Group for Cell Proliferation, Budapest, Hungary, 1992
3. Dou QP. Cyclins and cancer. Molecular and Cell Biology Program, University of Maryland at Baltimore, 1992
4. Dou QP, Keyomarsi K, Liang P, and Pardee AB. Aging, cell cycle and cancer. FASEB Summer Research Conferences, 1993
5. Dou QP. Regulation of cell proliferation in normal and cancer cells. Department of Pharmacology, University of Pittsburgh School of Medicine, 1993

6. Dou QP. Regulation of cell proliferation in normal and cancer cells. Pittsburgh Cancer Institute, 1993
7. Dou QP. Cyclins, cancer and apoptosis. Pittsburgh Cancer Institute scientific retreat, 1994
8. Dou QP. Cell cycle controls, cancer and programmed cell death. Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, People's Republic of China, 1994
9. Dou QP. Cell cycle, cancer and apoptosis. Institute of Occupational Medicine, Chinese Academy of Preventative Medicine, Beijing, People's Republic of China, 1994
10. Dou QP. Molecular mechanisms of cancer and apoptosis. Shandong Academy of Medical Sciences, Jinan, Shandong, People's Republic of China, 1994
11. Dou QP. Molecular controls of apoptosis: Involvement of cell cycle regulatory proteins. University of Pittsburgh Cancer Institute, Immunology Program, October 5, 1995
12. Dou QP. Minisymposium Speaker. Cleavage of retinoblastoma protein (RB) by a member of interleukin 1β-converting enzyme (ICE) family at the initiation of apoptotic execution. AACR 87th annual meeting, Washington, D.C., 1996
13. Dou QP. RB and breast cancer apoptosis. Basic and Translational Research on Breast Cancer Minisymposium, Magee-Womens Research Institute, Pittsburgh, PA, July 20, 1996
14. Dou QP. RB and apoptosis. Translational Research Seminar, UPCI, Pittsburgh, PA, September 28, 1996
15. Dou QP. Apoptosis control and cancer. Department of Pharmacology, University of Pittsburgh School of Medicine, February 7, 1997
16. Dou QP. Apoptosis control and cancer. *Cephalon*, Inc., March 11, 1997
17. Dou QP. RB and apoptosis. University of Pittsburgh Cancer Institute, Molecular Oncology Seminar Series, April 16, 1997
18. Dou QP. Activation of apoptotic death program in human cancer. H. Lee Moffitt Cancer Center & Research Institute at the University of South Florida, April 28, 1997
19. Dou QP. Invited Speaker. Retinoblastoma protein and the regulation of apoptosis. 7th SCBA International Symposium, Toronto, Canada, July 6-11, 1997.
20. Dou QP. Cell cycle and Apoptosis. University of Pittsburgh Cancer Institute, FAS-L Club, August 12, 1997.
21. Dou QP. Apoptosis regulation in breast cancer. Second Annual Pittsburgh Minisymposium on Basic and Translational Research in Breast Cancer, Center for Environmental and Occupational Health and Toxicology, University of Pittsburgh, August 16, 1997
22. Dou QP. Invited speaker. RB and apoptosis control. Center for Clinical Pharmacology, University of Pittsburgh School of Medicine, September 25, 1997
23. Dou QP. Invited Speaker. Targeting the Apoptotic Signaling Pathway in Human Cancer. Departments of Biochemistry & Molecular Biology and Microbiology & Immunology, University of North Texas Health Science Center at Fort Worth, September 29, 1997
24. Dou QP. Invited Speaker and Session Chairman. Putative roles of retinoblastoma protein in apoptosis. 2nd World Congress on Advances in Oncology, Athens, Greece, 16-18 October, 1997
25. Dou QP. Invited Speaker. Cell cycle checkpoint proteins as apoptosis therapeutic targets. NATO Advanced Research Workshop at H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Protein-Protein and Protein-Lipid Interactions in Signal Transduction: Use of Small Synthetic Molecules as probes and Therapeutic Agents, Clearwater Beach, Florida, December 5-9, 1997
34. Dou QP. Targeting ubiquitin/proteasom-mediated protein degradation pathway in human cancers. Research Progress Seminar Series at H. Lee Moffitt Cancer Center and Research Institute and University of South Florida, Tampa, Florida, October 29, 1998

35. Dou QP. Invited Speaker. Targeting ubiquitin/proteasom-mediated protein degradation pathway in human cancers. Department of Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, Florida, February 17, 1999
36. Dou QP. Invited Speaker. Bax degradation by the proteasome: a survival mechanism used by human cancer cells. Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, Tampa, Florida, October 15, 1999
37. Gao G and Dou QP. G1 phase-dependent Bcl-2 expression correlates with chemoresistance of human cancer cells. Oral presentation. 29th Annual Scientific Meeting of the International Society for Experimental Hematology, Tampa, FL, July 8-11, 2000
38. Smith DM and Dou QP. Green tea targets human tumor cell DNA synthesis and consequently induces apoptosis. Poster presentation. New Molecular Targets for Cancer Therapy, St. Petersburg Beach, FL, October 14-17, 2000
39. Dou QP. Invited Speaker. Proteasome inhibitors as novel anticancer drugs. Cancer Research and Biotechnology in the I-4 Corridor, Moffitt Cancer Center & Research Institute, Tampa, Florida, August 21, 2000

X. PATENTS

"Multicatalytic Protease (Proteasome) Inhibitors for Use as Anti-Tumor Agents", filled in on 12/16/97 (US, Ser. No. 60/069,804) and (International, WO 99/30707)

"Cell-Free Bax Degradation Assay: Application in Cancer Diagnosis, Prognosis and Treatment", filled on 11/9/99 (US, Ser. No. 60/186,895)

"A Calpain-Cleaved Bax Fragment, Bax/p18, with A Potent Cell Death-Inducing Ability: Implications in Cancer Therapy", filled on 3/16/2000

"Inhibition of Proteasome Activity In Vitro and In Vivo by Ester Bond-Containing Tea Polyphenols", filled on 4/27/2000

XI. ACADEMIC/PROFESSIONAL EXPERIENCE

A. Reviewer for Journal Manuscripts

Proceeding of National Academy of Sciences USA
Oncogene
Cancer Research
Molecular Pharmacology
Journal of Pharmacology & Experimental Therapeutics
Leukemia
International J. Oncology
J. Cell. Biochemistry
Natural Immunity
Molecular Biology Reports

Gene Therapy
Expert Opinion on Investigational Drugs
The Pittsburgh Undergraduate Review

B. Reviewer for Grant Applications

1996	Competitive Medical Research Fund (Ad Hoc Reviewer), University of Pittsburgh School of Medicine
1997	Competitive Medical Research Fund, University of Pittsburgh School of Medicine
1997	Central Research Development Fund, University of Pittsburgh
1998	National Science Foundation
1999-present	Grant Reviewer, Oncology Merit Review Subcommittee, the Veterans Affairs Medical Research Programs, US Department of Veterans Affairs, Veterans Health Administration (Spring and Fall)

C. Courses Taught at University of Pittsburgh

1993-1998	MS MIC 2355: Advanced Molecular Genetics. Lecture and Paper Discussion. 3 credits. 8-16 students
1993-1998	PHL 3510: Receptors and Signal Transduction. Lecture and Paper discussion. 3 credits. 10-15 students
1993-1998	2563: Cancer Pharmacology. Lecture. 3 credits. ~5 students
1993-1998	Medical Student Program: Problem-Based Learning Sessions. 8-10 students
1995	Medical Student Program: Pharmacology Conference. ~20 students
1997	Medical Student Program: Neoplasia and Neolastic Disease. 16 students
1996-97	The Pennsylvania Governor's School Program. 6-8 students
1997	Foundations of Biomedical Science. Small group conference. 3 credits. ~8 students

Courses Taught at University of South Florida

1999-2000	BCH 6411: Molecular Biology. Lecture. 3 credits. 25-30 students

D. Undergraduate and Graduate/Medical Student Supervision

1994	Chen Yu, Harvard University, ASPET undergraduate
1995	Peggy Lin, Penn State-Jefferson
1995	Bill Wang, California University of PA
1995	Vivian Lui, Department of Pharmacology, University of Pittsburgh School of Medicine, one lab rotation
1996	Toni A. Termin, Saint Vincent College, ASPET undergraduate
1996	Kirk E. Dineley, Department of Pharmacology, University of Pittsburgh School of Medicine, two lab rotations

1996-1999	Cheryl Fattman, Department of Pharmacology, University of Pittsburgh School of Medicine. Ph.D., Graduation with Honor
1997	Lachelle Sussman, University of New York at Buffalo, ASPET undergraduate
1997	Ana Vasquez, Interdisciplinary Biomedical Graduate Program, University of Pittsburgh School of Medicine, one lab rotation
1998	Kristin S Morrow, Department of Biology University of South Florida, master graduate student
1998-present	Yaser S. Bassel, University of South Florida College of Medicine, medical student
1998-present	Jason A. Evangelista, University of South Florida College of Medicine, medical student
1998-present	Joseph J. Kavanagh, University of South Florida College of Medicine, medical student
1998-present	Alexander Paloma, University of South Florida College of Medicine, medical student
1998-present	Gregory A. Russell, University of South Florida College of Medicine, medical student
1999-present	David Smith, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation and Ph.D. candidate student in my lab
1999	Lisa Smith, Department of Biology University of South Florida, undergraduate student
1999	Jessica Hu, Harvard University, undergraduate student
1999	Daniel Lorch, University of Florida, undergraduate student
1999	Sun Hee Rim, Hillsborough High School, student
1999	Alvin Jones, Land O'Lakes High School, student
1999	Kristie Main, Department of Biochemistry & Molecular Biology, University of South Florida College of Medicine, one lab rotation
1999-2000	Kenyon Daniel, Department of Biology University of South Florida, undergraduate student. Research for Honor's Thesis
2000	Lisa Smith, Moffitt Summer Intern, Department of Biology University of South Florida, undergraduate student
2000	Marie Bosley, Project LINK (Leaders In New Knowledge) Student and a Moffitt Summer Intern, Department of Biology University of South Florida, undergraduate student

E. Theses/ Dissertation or Comprehensive Examination Committees

Ph.D. Dissertation Committees

1995	Kirti G. Goyal, Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine (Advisor: Dr. Leaf Huang)
1997	Jie-Gen Jiang, Pathology, University of Pittsburgh School of Medicine (Advisor: Dr. Reza Zarnegar), graduated in 12/97
1997-1998	Marni Brisson, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. Leaf Huang)
1997-1998	Donald Schwartz, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. John Lazo)

1996-1998 Robert Rice, Pharmacology, University of Pittsburgh School of Medicine (Advisor: Dr. John Lazo)

Comprehensive Examination Committees

1994 Xiang Gao, Pharmacology, University of Pittsburgh School of Medicine
1995 Jeff Phillips, Pharmacology, University of Pittsburgh School of Medicine
1995 Chialin Cheng, Pharmacology, University of Pittsburgh School of Medicine
1995 Cheryl Fattman, Pharmacology, University of Pittsburgh School of Medicine
1996 Marni Brisson, Pharmacology, University of Pittsburgh School of Medicine

F. Research Associates and Others

2000-present Sherry Zhong, Research Assistant
2000-present Aslamuzzaman Kazi, Ph.D., Research Associate
2000-present Hongwei Wang, Research Assistant
2000-present Kenyon Daniel, Research Assistant

1998-2000 Sangkil Nam, Ph.D., Research Associate
2000 Gen Wang, Ph.D., Research Associate
1999-2000 Xiaoxia Zhang, M.S., Research Assistant
1998-2000 Gui Gao, Ph.D., Research Associate
1998-2000 Benyi Li, M.D., Research Associate
1998-1999 Roland Cooper, Ph.D., Research Associate
1998 Jieliu Tang, Ph.D., Research Associate
1994-1998 Bing An, Research Associate
1996-1998 Terence F. McGuire, Ph.D., Instructor
1997-1998 Yibing Peng, M.S., Research Assistant
1995-1996 Jia-Rui Jin, Visiting Scholar
1996 Leilei Zhang, Visiting Scholar

G. Committee Assignments

Department of Pharmacology, University of Pittsburgh School of Medicine

1993-1998 Comprehensive Examination Committee, Department of Pharmacology, University of Pittsburgh School of Medicine
1994-1998 Committee of Graduate Studies, Department of Pharmacology, University of Pittsburgh School of Medicine
1994-1998 Chairman of Graduate Evaluations, Department of Pharmacology, University of Pittsburgh School of Medicine
1995 NIH Predoctoral Training Grant Selection Committee
1997 Director of Departmental Seminar Program

University of Pittsburgh School of Medicine

1996-1997 Competitive Medical Research Fund Review Committee (Ad Hoc Reviewer), University of Pittsburgh School of Medicine

1997 The Graduate Progress Evaluation Committee, University of Pittsburgh School of Medicine

1997 Central Research Development Fund, University of Pittsburgh

H. Service

Tours for Drug Discovery Program (April, September 6, 1999)
Faculty Reviewer for Applicants to Moffitt and USF
Advisor for Project LINK (Leaders In New Knowledge) Student
Advisor for Moffitt Summer Interns
Advisor for Undergraduate Student Honor's Thesis Research

XII. GRANT SUPPORT

A. Completed support

American Cancer Society Institutional Research Grant. Cyclins, transcription and defective growth control in cancer. Principal Investigator: Qing Ping Dou. 10/01/93-06/30/95. Total Direct Costs: $10,000

An Agreement with Beth Israel Hospital. Molecular Biology of Aging. Principal Investigator: Jeanne Y. Wei. 1994. Total Direct Costs: $10,000

NIH/ Harvard Subcontract. Molecular Biology of G1/S Regulation in Murine Cells. Principal Investigator: Arthur B. Pardee. 07/01/93-06/30/96. Total Direct Costs: $82,040; Total Indirect Costs: $38,239

NIH Shannon Award. Functions of RB-protease(s) in apoptosis. Principal Investigator: Qing Ping Dou. 09/15/95-08/31/97 (replaced by R29 on 04/14/96). Total Direct Costs: $80,000; Total Indirect Costs: $20,000

UPCI Breast Cancer Pilot Grant. Induction of p53-independent apoptosis and treatment of human breast cancer. Principal Investigator: Qing Ping Dou. 03/15/96-09/30/97. Total Direct Costs: $20,000; Total Indirect Costs: $10,200

B. Present support

NIH FIRST Award. Functions of RB-protease(s) in apoptosis. Principal Investigator: Qing Ping Dou (50%). 04/15/96-02/28/01. Total Direct Costs: $349,996; Total Indirect Costs: $178,298

NIH R01 subcontract (from University of Pittsburgh). Growth Inhibition by IL-2 of IL2R+ oral carcinomas. Principal Investigator: Qing Ping Dou (10%). 04/01/98-03/31/01. Total Direct Costs: $105,270; Total Indirect Costs: $44,740

H. Lee Moffitt Start-Up Fund. Principal Investigator: Qing Ping Dou. 04/30/98-04/29/01. Total Direct Costs: $235,000

Department of the Army Advanced Cancer Detection Center Research Grant (Moffitt). Principal Investigator: Q. Ping Dou. 01/01/00-12/31/00. Total Direct Costs: $114,773

C. Pending support

NIH R01. Proteasome as A Target for Cancer Therapy. Principal Investigator: Q. Ping Dou (25%). 12/01/00-11/30/05. Total Direct Costs: $875,000; Total Indirect Costs: $221,062

DOD IDEA. Ester Bond-Containing Tea Polyphenols Potently Inhibit the Proteasome Activity: Implication in Breast Cancer Prevention and Treatment. Principal Investigator: Q. Ping Dou (25%). 03/01/01-02/29/04. Total Direct Costs: $300,000; Total Indirect Costs: $135,000

American Institute for Cancer Research. Tea Polyphenols Target Proteasome-Mediated Bax Degradation Pathway: Significance in Prostate Cancer Prevention and Treatment. Principal Investigator: Q. Ping Dou (15%). 02/01/01-01/31/03. Total Direct Costs: $150,000; Total Indirect Costs: $15,000

NIH R21. Calpain as A Target for Cancer Drug Discovery. Principal Investigator: Q. Ping Dou (25%). 04/01/01-03/31/03. Total Direct Costs: $200,000; Total Indirect Costs: $90,000

NIH R03. Tea Targeting Proteasome: A Role in Cancer Prevention. Principal Investigator: Q. Ping Dou (10%). 07/01/01-06/30/03. Total Direct Costs: $100,000; Total Indirect Costs: $45,000